UNIVERSIDADE ESTADUAL PAULISTA – UNESP

CÂMPUS DE JABOTICABAL

RELAÇÕES DE AMINOÁCIDOS EM DIETAS DE SUÍNOS ALIMENTADOS ATRAVÉS DO SISTEMA DE ALIMENTAÇÃO DE PRECISÃO

Pedro Righetti Arnaut

Mestre em Zootecnia

Médico Veterinário

2024

UNIVERSIDADE ESTADUAL PAULISTA – UNESP

CÂMPUS DE JABOTICABAL

RELAÇÕES DE AMINOÁCIDOS EM DIETAS DE SUÍNOS ALIMENTADOS ATRAVÉS DO SISTEMA DE ALIMENTAÇÃO DE PRECISÃO

Discente: Pedro Righetti Arnaut

Orientador: Prof. Dr. Luciano Hauschild

Coorientadora: Dra. Aline Remus

Tese apresentada à Faculdade de Ciências Agrárias e Veterinárias – Unesp, Câmpus de Jaboticabal, como parte das exigências para a obtenção do título de Doutor em Zootecnia.

2024

FICHA CATALOGRÁFICA

A745r

Arnaut, Pedro Righetti
Relações de aminoácidos em dietas de suínos alimentados através do sistema de alimentação de precisão / Pedro Righetti Arnaut. -- Jaboticabal, 2024
139 p. : il., tabs., fotos, 4 v.

Tese (doutorado) - Universidade Estadual Paulista (UNESP), Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal
Orientador: Luciano Hauschild
Coorientadora: Aline Remus

1. Desafio sanitário. 2. Categorias de crescimento. 3. Exigências dinâmicas. I. Título.

Sistema de geração automática de fichas catalográficas da Unesp. Biblioteca da Universidade Estadual Paulista (UNESP), Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal. Dados fornecidos pelo autor(a).

Essa ficha não pode ser modificada.

CERTIFICADO DE APROVAÇÃO

UNIVERSIDADE ESTADUAL PAULISTA

Câmpus de Jaboticabal

CERTIFICADO DE APROVAÇÃO

TÍTULO DA TESE: RELAÇÕES DE AMINOÁCIDOS EM DIETAS DE SUÍNOS ALIMENTADOS ATRAVÉS DO SISTEMA DE ALIMENTAÇÃO DE PRECISÃO

AUTOR: PEDRO RIGHETTI ARNAUT
ORIENTADOR: LUCIANO HAUSCHILD
COORIENTADORA: ALINE REMUS

Aprovado como parte das exigências para obtenção do Título de Doutor em Zootecnia, pela Comissão Examinadora:

gov.br Documento assinado digitalmente
LUCIANO HAUSCHILD
Data: 27/08/2024 08:00:28-0300
Verifique em https://validar.iti.gov.br

Prof. Dr. LUCIANO HAUSCHILD (Participaçao Virtual)
Departamento de Zootecnia / FCAV UNESP Jaboticabal

gov.br Documento assinado digitalmente
LUAN SOUSA DOS SANTOS
Data: 27/08/2024 18:59:23-0300
Verifique em https://validar.iti.gov.br

Prof. Dr. LUAN SOUSA DOS SANTOS (Participaçao Virtual)
Universidade Federal do Mato Grosso do Sul (UFMS) / Campo Grande/MS

gov.br Documento assinado digitalmente
URBANO DOS SANTOS RUIZ
Data: 02/09/2024 08:25:36-0300
Verifique em https://validar.iti.gov.br

Prof. Dr. URBANO DOS SANTOS RUIZ (Participaçao Virtual)
Departamento de Zootecnia / ESALQ USP Piracicaba/SP

Profa. Dra. CHEILA ROBERTA LEHNEN (Participaçao Virtual)
Departamento de Zootecnia / Universidade Estadual de Ponta Grossa (UEPG) - Ponta Grossa/PR

gov.br Documento assinado digitalmente
CHEILA ROBERTA LEHNEN
Data: 27/08/2024 09:32:17-0300
Verifique em https://validar.iti.gov.br

Profa. Dra. NILVA KAZUE SAKOMURA (Participaçao Virtual)
Departamento de Zootecnia / FCAV UNESP Jaboticabal

gov.br Documento assinado digitalmente
NILVA KAZUE SAKOMURA
Data: 12/09/2024 15:37:44-0300
Verifique em https://validar.iti.gov.br

Jaboticabal, 26 de agosto de 2024

DADOS CURRICULARES DO AUTOR

PEDRO RIGHETTI ARNAUT, nascido em São Paulo – SP no dia 28 de abril de 1993, ingressou no curso de Medicina Veterinária na Universidade Federal de Viçosa no ano de 2011 e se graduou no ano de 2018. Durante a graduação, fez estágio nas áreas de suinocultura, avicultura de corte e postura e realizou projetos de pesquisa. Do ano de 2014 a 2015, foi contemplado com uma bolsa integral do programa Ciências sem Fronteiras (governo de Dilma Rousseff) e fez curso de inglês e matérias de ciência animal na Indiana University e University of Kentucky, respectivamente. Ao término da sua estadia na University of Kentucky seu nome foi incluído na Dean's List, prêmio interno da universidade concedido aos alunos que obtiveram rendimento escolar anual igual ou superior a 90%. Em julho de 2018, se formou em Medicina Veterinária e foi aprovado no processo seletivo de Pós-Graduação em Zootecnia do Departamento de Zootecnia da Universidade Federal de Viçosa sob orientação da Profa. Dra. Melissa Izabel Hannas. Em Julho de 2020 se formou mestre e foi aprovado no processo seletivo de Pós-Graduação em Zootecnia do Departamento de Zootecnia da Universidade Estadual Paulista "Júlio de Mesquita Filho" – Jaboticabal sob orientação do Prof. Dr. Luciano Hauschild. Em 2023 foi contemplado com uma bolsa de doutorado sanduíche a ser realizada no Sherbrooke Research and Development Centre – Agriculture and Agri-food Canada. Durante o doutorado sanduíche, ganhou o prêmio de melhor projeto de pesquisa apresentado por alunos de doutorado no 2023-ASAS-CSAS Annual Meeting.

AGRADECIMENTOS

Aos meus pais (Elonyr e Flamarion) e meus irmãos (Laura e Lucas) por sempre me apoiarem e estarem ao meu lado em todas as decisões da minha vida. Por terem me incentivado a estudar mesmo quando eu não queria. Por terem me mostrado o valor do trabalho e da perseverança. Por me ensinarem que, quando a vida fecha todas as portas e tranca todas as janelas, não temos outra opção senão continuar. Uma hora tudo melhora. Essa tese é para vocês!

À minha companheira de vida, Graziela, por estar ao meu lado nos meus piores e melhores momentos. Por ter me mostrado que a vida pode ser mais leve e tranquila mesmo quando nossos dados não estão fazendo sentido. Por ter me dado o privilégio de construir uma família linda e amorosa com nossas filhas de quatro patas (Amora, Nanu e Cacau). Por sempre ter me apoiado, incentivado, alegrado e acreditado no meu potencial quando eu não acreditei. Essa tese também é para você!

Ao meu orientador, Prof. Dr. Luciano Hauschild, por todos os ensinamentos e por acreditar no meu potencial. Por ter me dado a oportunidade de trabalhar com a alimentação de precisão e por incentivar meu crescimento profissional. Muito obrigado!

À minha coorientadora, Dra. Aline Remus, por ter me dado o privilégio de ser seu orientado. Obrigado pelos ensinamentos, companheirismo e suporte durante o doutorado. Obrigado por ter nos mostrado a dimensão do profissional que poderíamos nos tornar. A alimentação de precisão está em boas mãos!

To Dr. Candido Pomar, for showing me that there is always something we can improve in our research. For encouraging my critical thinking and for contributing with ideas to this thesis. “The thesis is important, but to have a walk in the woods is more important”. Right?! Thank you, Candido!

Aos pós-doutores Danilo e Diego por terem contribuído com o meu aprendizado e me auxiliado em todas as etapas do doutorado.

Aos colegas de pós-graduação do Laboratório de Estudos em Suinocultura: Ismael, Manoela, Cleslei, Marllon, Lucas, Joseane e Alícia por terem contribuído

para a condução dos experimentos e pelo companheirismo durante o doutorado. Não teria sido fácil sem vocês.

A huge “Merci beacoup!” to Sophie Horth for teaching me to double-check the double-checks. Sophie, thank you for being by my side in the experiments we conducted in the AAFC. The trials would not have been easy without you! Hope to see you again and drink an ice-cold rum and coke! Merci, Sophie!

To Jean-Philippe Brousseau for his assistance in the trials and for teaching me his thorough laboratory skills.

To Steve Methot for his assistance with the statistical analysis and for showing me what SAS is capable of doing! Thank you, Steve.

Ao Zé (José Segecic), funcionário do Laboratório de Estudos em Suinocultura, por ter auxiliado na condução do experimento realizado no Brasil com muito humor e companheirismo.

Aos estagiários do Laboratório de Estudos em Suinocultura Amanda, Giulia, Gabriela, Isadora, Jaqueline, Maria Beatriz e Isabella por terem auxiliado na condução do experimento conduzido no Brasil.

To the AAFC Swine Complex employees, especially to Mélanie, Joel, Edouard, the two Mikes, Judy, July, Kassandra, Sarah and Dominic for their outstanding work and professionalism in the trials conducted in Canada. Take it easy with the Tim Hortons’ muffins, guys!

À Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, número do processo 2018/15559-7 e 2019/10843-1), O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001. ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, número do processo 408287/2021-7 e 442675/202-2), à Evonik Operations GmbH e Seara Alimentos – JBS Foods Brasil por financiarem os projetos e bolsa de doutorado.

A todos que fizeram parte da minha jornada profissional do início da graduação até os dias de hoje e que contribuíram para a construção do profissional que sou. Obrigado!

SUMÁRIO

CERTIFICADO DA COMISSÃO DE ÉTICA NO USO DE ANIMAIS

(CAPÍTULO 2)

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Câmpus de Jaboticabal

CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS

C E R T I F I C A D O

Certificamos que o projeto de pesquisa intitulado **"Nutrição de precisão e suplementação de aminoácidos funcionais para suínos sob desafio ambiental"**, protocolo nº 0607/22**,** sob a responsabilidade do Prof. Dr. Luciano Hauschild, que envolve a produção, manutenção e/ou utilização de animais pertencentes ao Filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da lei nº 11.794, de 08 de outubro de 2008, no decreto 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), da FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS, UNESP - CÂMPUS DE JABOTICABAL-SP, em reunião ordinária de 18 de maio de 2022.

Vigência do Projeto	01/03/2022 a 01/08/2022
Espécie / Linhagem	Suínos
Nº de animais	120
Peso / Idade	10 a 75 kg
Sexo	Fêmeas
Origem	Granja comercial parceira

Jaboticabal, 18 de maio de 2022.

Profª Drª Paola Castro Moraes
Vice-Coordenadora – CEUA

Faculdade de Ciências Agrárias e Veterinárias
Via de Acesso Prof. Paulo Donato Castellane, s/n CEP 14884-900 - Jaboticabal - SP - Brasil
Tel. 16 3209-7100 - www.fcav.unesp.br

CERTIFICADO DA COMISSÃO DE ÉTICA NO USO DE ANIMAIS

(CAPÍTULO 3)

Comité institutionnel de protection des animaux
Centre de recherche et de développement de Sherbrooke

FORMULAIRE D'APPROBATION DES MÉTHODES EXPÉRIMENTALES SUR LES ANIMAUX

Numéro CIPA : 617 **Évaluation du mérite scientifique** OUI ☒ NON ☐ DATE : 2019-05-01

Projet réalisé par (organisme subventionnaire ou programme de subvention) : FAPESP – Project 2018/15559-7

Évaluation CIPA DATE: 17 janvier 2023

Titre du projet : Response to different Threonine ratios of growing pigs fed daily tailored diets

Titre court du projet (1 à 2 mots maximum) : Calibration Mangeoires

Mots-clés: (Choisir parmi les mots clés recommandés par le CCPA selon l'**annexe A**) : étude en science fondamentale, contention physique (de courte ou de longue durée), manipulation, examen physique

Résumé vulgarisé du projet (Objectif): (Brève description en termes simples [40 mots]) :
Le but de cette étude est de tester de régies alimentaires avec trois profils des acides aminés. Les profils auront quatre niveaux de thréonine pour des porcs en croissance dans un système de nutrition de précision.

Résumé des actions posées sur les animaux vivants incluant quantité/volume/poids des échantillons :

Soixante (60) porcs (30 mâles castrés et 30 femelles) du même génotype de haute performance, avec environ 18 kg de poids corporel seront expédiés de la section pouponnière à la croissance/finition au complexe porcin du Centre de Recherche AAC- Sherbrooke. Une puce électronique pour donner accès aux mangeoires automatiques et un tag d'identification seront installés à l'oreille à leur arrivée à la croissance/finition. Cinquante porcs (5 mâles et 5 femelles par traitement) seront sélectionnés pour la phase animale. Les porcs seront logés dans 1 parc commun à la croissance/finition avec 5 mangeoires automatiques (JYGA). Il y aura une période d'adaptation (14 jours) des porcs à l'environnement et aux mangeoires automatiques. Il aurait 5 traitements (contrôle phase, nutrition de précision avec 80, 100, 120 ou 140% du ratio idéel Thréonine :Lysine). La phase de croissance qui suivra durera 28 jours et servira à mesurer la croissance et composition corporelle des porcs. Les porcs seront pesés 12 fois tout au long de l'expérimentation et adaptation (2 fois par semaine). Des échantillons de sang (20 ml porc/jour de prélèvement) seront collectés dans des tubes de type vacutainers au jour 1, 7 et au jour 28 dans la veine jugulaire par ponction veineuse. Les porcs seront scannés avec le Lunar deux fois (au jour 1 et 28) pour évaluer la composition corporelle. Le sevoflurane (7%) sera utilisé pour induire l'anesthésie et l'isoflurane (5%) pour la maintenir. Un tranquillisant (Stressnill) sera injecté (IM) dans les rares cas de porcs récalcitrants. À la fin de l'essai, les porcs peuvent être commercialisés.

Numéros de projets CIPA en référence à un ou des protocoles reliés, inscrivez NIL sinon : NIL

			OUI	NON	S/O	À venir
Chercheur responsable :	Aline Remus	*Formation théorique CCPA	☒	☐	☐	☐
Chercheurs collaborateurs du CRDS :	1. Candido Pomar	*Formation théorique CCPA	☒	☐	☐	☐
	2.	*Formation théorique CCPA	☐	☐	☐	☐
	3.	*Formation théorique CCPA	☐	☐	☐	☐
	4.	*Formation théorique CCPA	☐	☐	☐	☐

Autres chercheurs collaborateurs impliqués : (Noms et institutions)		OUI	NON	S/O	À venir
1. Luciano Hauschild, Unesp - Brésil	*Formation théorique CCPA	☐	☐	☒	☐
2.	*Formation théorique CCPA	☐	☐	☐	☐

3.	*Formation théorique CCPA	☐	☐	☐	☐
4.	*Formation théorique CCPA	☐	☐	☐	☐

Nom, fonction et formation académique pertinente des personnes manipulant les animaux : (Personnel de recherche, étudiants 1er, 2e et 3e cycle, étudiants postdoctoraux, animaliers, etc.)		**OUI**	**NON**	**S/O**	**À venir**
1. Pedro Righetti Arnaut, étudiant au 3e cycle de Sciences Animales	*Formation théorique CCPA	☐	☐	☐	☒
2. Sophie Horth, Master en Science Animal, Assistante de recherche	*Formation théorique CCPA	☒	☐	☐	☐
3. Annie Turcotte, Master en Microbiologie, Assistante de recherche	*Formation théorique CCPA	☒	☐	☐	☐
4.	*Formation théorique CCPA	☐	☐	☐	☐

* La formation CCPA est obligatoire pour les gens qui manipulent les animaux ainsi que leur(s) superviseur(s)

Si le projet ne nécessite pas l'utilisation d'animaux, passez au point 13

Phase animale	Date de début :	2023-03-27
	Date de fin :	2023-05-12
Secteur	Porcs	☒
	Bovins laitiers	☐

Projet réalisé en tout ou en partie en-dehors du Centre de R & D de Sherbrooke
(Si oui, indiquez le lieu)

☐ Oui
☒ Non

Projet réalisé à des fins réglementaires (études cliniques) **pour l'obtention d'une approbation par Santé Canada ou d'autres organismes réglementaires.** (Si oui, répondez aux 2 questions suivantes)

☐ Oui
☒ Non

A- Le projet a été planifié selon les lignes directrices réglementaires les plus récentes

☐ Oui
☐ Non

Justifiez :

B- Le nombre d'animaux utilisés ne dépasse pas le nombre exigé par les autorités réglementaires

☐ Oui
☐ Non

Justifiez :

1. Catégorie de techniques invasives en expérimentation animale

Choisir à quelle catégorie du CCPA appartient votre projet en vous référant à l'**annexe C**. C

A. Expériences avec la plupart des invertébrés ou avec des prélèvements de tissus vivants
B. Expériences causant peu ou pas d'inconfort ou de stress
C. Expériences causant un stress mineur ou une douleur de courte durée
D. Expériences causant une détresse ou un inconfort modéré(e) à intense
E. Procédures causant de la douleur intense égale ou au-dessus du seuil de tolérance de la douleur chez des animaux éveillés non anesthésiés

- Commentaire (facultatif) :

2. But de l'utilisation des animaux

Choisir une catégorie de 0 à 5 selon l'**annexe D**. 1

0. Colonie d'élevage ou stock
1. Études en sciences fondamentales portant sur des structures ou des fonctions essentielles
2. Études médicales, notamment dans le domaine de la médecine vétérinaire, portant sur des maladies ou des troubles chez l'humain ou l'animal
3. Essais réglementaires de produits pour la sécurité des humains ou des animaux ou la protection de l'environnement
4. Études de développement de produits ou de dispositifs à l'usage de la médicine humaine ou vétérinaire
5. Enseignement post-secondaire ou formation en cours d'emploi

➢ Commentaire (facultatif) :

3. Type d'expérimentation

Précisez si les interventions sont aiguës, au niveau du laboratoire, chroniques ou mixtes selon la définition du CCPA : (Choisir A, B, C ou M) | C

A. Aigu : toute étude au cours de laquelle un animal est euthanasié de façon humanitaire *dans le seul but de récolter certains tissus.*

B. Laboratoire : expérience *in vitro* et/ou échantillons récoltés à l'abattoir chez des animaux d'origine inconnue.

C. Chronique : toute autre étude où l'animal n'est pas euthanasié.

M. Mixte : étude ayant une phase animale pour collecter des données et qui se termine par l'euthanasie de l'animal (avec ou sans prélèvement de tissus).

4. Utilisation des animaux : Espèce

A. Espèces animales et nombre exact d'animaux enrôlés au total y compris les animaux de réserve (*back up*), les animaux sentinelles et figurants.

	Nb
Porcs croissance	50
Back up	10
TOTAL	60

B. L'expérience nécessite-t-elle une contention (Immobilisation d'un animal par divers moyens pour restreindre ses activités) autre que celle utilisée dans la régie normale des animaux? ☒ Oui ☐ Non

Si oui, **précisez :** (Moyen, durée, etc.)

Lors de l'anesthésie du porc, l'animal est contentionné avec un panneau (~5-10 min)
Lors de la récolte de sang, l'animal est mis en cage (5-10 min)

C. Nommez tous les types d'hébergement des animaux et pendant combien de temps ils y seront logés
(cage de mise-bas, parc en groupe de (combien ?) individus, cage métabolique, logette, hutte, stalle, enclos, étable de biosécurité II, etc.)

Parc en groupe de 60 animaux pendant les 14 jours d'adaptation, parc en group de 50 pendant les 28 jours d'expérimentation

D. Enrichissement du milieu : qui va au-delà des besoins physiques, physiologiques et psychologiques fondamentaux.

Les animaux seront-ils privés de l'enrichissement habituellement offert en régie normale? ☐ Oui ☒ Non

Nommez un minimum de 2 enrichissements parmi ceux-ci habituellement offert en régie normale :

Porc (exemples mis à jour en 2022) : Contact visuel ou physique avec porc/fenêtre dans cloison, jouet, plancher solide ou tapis caoutchouc, exercice périodique ou régulier, musique, brossage/lavage, présence humaine, zone cafétéria, logement en groupe, période d'adaptation avant changement, repos additionnel avant abattoir/encan, heures de transport adaptées aux saisons, exutoire sexuel pour verrat.

Bovin : Marche 2 fois/jour vers le salon de traite, brossage/lavage, musique, présence humaine, logement en groupe, jouet, aire d'exercices avec brosse, repos additionnel avant encan ou abattoir, litière améliorée, autres à venir.

RÉPONSE : jouets, exercice périodique ou régulier, présence humaine, logement en groupe, période d'adaptation avant changement

Si les animaux sont privés de l'enrichissement habituellement offert en régie normale, justifiez-en la raison et indiquez quels deux autres seront offerts en contrepartie (ex. période d'entraînement avant projet, exercice supplémentaire, présence humaine additionnelle, nouveau jouet; porc: bauge, litière, accès au fourrage ou aliment autre que moulée; bovin: air conditionné, fenêtres et microphone).

RÉPONSE :

5. Remplacement, réduction et raffinement (les Trois R)

A. Est-ce que l'utilisation de méthodes alternatives (culture de tissu, prélèvement de matériel biologique ou autres) qui ne nécessitent pas d'animaux vivants peut s'appliquer?

☐ Oui
☒ Non

Justifiez : Nous avons utilisé des alternatives in silico antérieures à cette expérience, et nous avons atteint ses limites. Les résultats ne peuvent pas être obtenus in vitro une fois que la réponse protéique du corps entier ne peut pas être simulée dans cet environnement.

B. Est-ce l'évaluation du nombre d'animaux demandés a été effectuée par un statisticien lors de l'élaboration du projet ou lors de l'évaluation scientifique par les pairs (puissance statistique)?

☐ Oui
☒ Non

Si oui, **veuillez fournir une référence** et si non, **justifiez :**

Pour le paramètre principal, donnez la moyenne et l'écart type associé à cette moyenne :

Porcs de 25-45 kg de poids corporelle - Dépôt de protéines : 143 g/j, Erreur standard maximale : 8.3 (Remus et al., 2019a).

En accord avec les résultats obtenus dans les expériences précédentes dans notre laboratoire, ce nombre nous permettra de mettre en évidence des différences significatives pour l'industrie porcine. Le nombre d'unités expérimentales est le même que dans les expériences précédentes similaires.

Références :

Andretta I, Pomar C, Rivest J, Pomar J and Radünz J 2016. Precision feeding can significantly reduce lysine intake and nitrogen excretion without compromising the performance of growing pigs. animal, 1-11.

Remus, A., Hauschild, L., Corrent, E., Létourneau-Montminy, M.-P., & Pomar, C. (2019). Pigs receiving daily tailored diets using precision-feeding techniques have different threonine requirements than pigs fed in conventional phase-feeding systems. Journal of animal science and biotechnology, 10(1), 16. doi:10.1186/s40104-019-0328

Remus, A., Pomar, C., Perondi, D., Gobi, J., da Silva, W., de Souza, L., & Hauschild, L. J. L. S. (2019). Response to dietary methionine supply of growing pigs fed daily tailored diets or fed according to a conventional phase feeding system. Livestock Science, 222, 7-13. doi:https://doi.org/10.1016/j.livsci.2019.02.006

C. Est-ce que la technique utilisée réduit au minimum le niveau de détresse imposée aux animaux? ☒ Oui ☐ Non

Justifiez : : Le protocole d'anesthésie au gaz Sevoflurane et Isoflurane a été essayé avec succès dans des projets antérieurs. Un tranquillisant (Stressnill) injecté peut aussi s'appliquer pour les porcs nerveux.

6. <u>Pour chacune des catégories d'animaux, décrivez les points limites de l'expérimentation</u>

A. Types et fréquence des observations : La consommation d'aliment et l'état général des animaux seront vérifiés au moins 2 fois par jour. Les animaux seront sous surveillance continue lors de l'anesthésie, pendant le scan et pendant le réveil.

B. Choix des points limites : Fièvre (> 40 oC), infection, absence de consommation d'aliment pendant plus d'un jour, diarrhée, problèmes respiratoires, problèmes majeurs de boiterie ou de locomotion.

C. Choix des interventions : Consultation du vétérinaire traitant pour établir un plan d'action pour tout traitement pharmaceutique pour les interventions médicales sur les animaux. Les animaliers vont surveiller quotidiennement les animaux. Si nécessaire, l'utilisation d'un analgésique anti-inflammatoire (Anafen) pourrait être autorisé pour des raisons spécifiques par la chercheuse si si les points limites, décrits ci-dessus, sont atteints. Aucun antibiotique ne sera autorisé, les animaux ayant besoin de ce médicament seront retirés de l'essai afin qu'ils puissent être traités.

D. Qui a la responsabilité de l'observation/surveillance des animaux?

Nom et titre : Équipe de la porcherie sous la responsabilité de la contremaître Mélanie Turcotte, Pedro Righetti Arnaut étudiant au 3e cycle de Sciences animales, Aline Remus, chercheure responsable, Sophie Horth, assistante de recherche, Annie Turcotte, assistante de recherche.

7. <u>Devenir des animaux utilisés dans le projet</u>

Est-ce que les animaux sont sacrifiés dans le cadre du projet? S/O ☐ Oui ☐ Non ☒

Si **oui**, est-ce que la méthode d'euthanasie est conforme aux normes acceptées par le CCPA? Oui ☐ Non ☐

(Décrire la méthode)

Si **non-conforme**, décrivez et justifiez la méthode qui sera utilisée :

Précisez le devenir des animaux vivants à la fin du projet en incluant les animaux de réserve et figurants : (ex. Retour dans le troupeau, abattage à la ferme, abattage commercial, vente, réforme) : vente

8. <u>Interventions qui seront pratiquées chez les animaux vivants</u>

(Veuillez mentionner le titre ou le numéro des procédures normalisées de fonctionnement (PNF) reliées aux interventions. (<u>PNF section porc</u> /<u>PNF section bovin laitier</u>)

A.

						<u>Numéro ou Titre de PNF</u>
Jeûne	X	Nombre d'heures :	8-12h	Âge des animaux :	56 – 98 jours	
Injections :	☐	sous-cutanée				
	☐	intra musculaire				
	☐	intra veineux				
	☐	autre (précisez) :				
Prélèvements :	☐	lait	☐	lait aseptique		
	☒	sang	☐	sang aseptique		Prélèvements Sanguins
			☐	sang grand volume		
	☐	fèces				
	☐	urine				
	☐	salive				
	☐	liquide ruminal				
	☐	biopsie mammaire				
	☐	autre (précisez) :				
Infusion	☐	Incubation ruminale	☐			
Infection	☐					
Cathéter	☐	type (précisez) :				
Canulation	☐					
Pesée	☒					Pesée Des Porcs
Gavage	☐					
Gras dorsal	☐					
Euthanasie	☐					
Numérisation	☒	(LUNAR)				CRDBLP-P-6017
Uniformisation de la portée hors régie normale	☐					
Ingestion/Insertion de puce électronique	☒					Identification des porcs par tag

Autre(s) ☐ (précisez) :

Chirurgie **(incluant les biopsies)** ou toutes autres procédures pouvant nécessiter une anesthésie ou une analgésie. Oui ☒ Non ☐

Si **oui**, répondez aux questions suivantes : Si **non**, passez au point 8-B

- Laquelle? Rayon X (Lunar) 2 fois/animal CRDBLP-P-6017

Description détaillée des procédures chirurgicales ou de toute autre procédure pouvant nécessiter une anesthésie ou une analgésie (inclure une copie des références, le cas échéant) :

- Quel est le nombre maximum prévu par jour? 13
- Est-ce que l'animal sera sous anesthésie locale? Oui ☐ Non ☒
- Est-ce que l'animal sera sous anesthésie générale? Oui ☒ Non ☐
- Si aucune anesthésie ou analgésie n'est utilisée, **justifiez** :

- Est-ce que la personne qui fera cette procédure l'a déjà faite auparavant? Oui ☒ Non ☐

Si **oui**, inscrivez le nom et la formation de la personne :

Sophie Horth, formation suivie en 2014, et ensuite, plusieurs projets avec scan ont été réalisés.

Personnel de la porcherie : formation faite lors de projets antérieurs

Si **non**, inscrivez le nom et le titre de la personne responsable qui formera :

Et remettez le formulaire *Méthode et Attestation de formation complétée 2019* avant que la personne formée ne débute les chirurgies de manière autonome.

- Est-ce que des soins post-opératoires seront administrés? Oui ☒ Non ☐
- **Si oui**, qui seront les personnes responsables (noms et titres)?

Décrivez le protocole de soins post-opératoires incluant la fréquence des observations :
Récupération de l'anesthésie avant réintégrer le groupe : le porc doit être réveillé et capable de marcher jusqu'à l'enclos avant d'être remis avec le groupe.
Observation du comportement des animaux pendent le réveil : respiration normale, coloration, et prise de température dans le cas où le porc prendre plus de 15 minutes pour ce réveiller après la fin de la procédure de l'anesthésie.

B. Autre(s) intervention(s) (précisez) : Oui ☐ Non ☒

Si oui, indiquez la fréquence, la durée et la quantité pour chaque type d'intervention

C. Donnez le nom, la dose, la fréquence et la voie d'administration de tous les médicaments des catégories suivantes utilisés lors des procédures:

Tranquillisant : Stressnill i.m. 2.2 mg/kg poids vif, 1 fois /porc/scan si besoin via intramusculaire.

Pré-anesthésique :

Anesthésique : Sevoflurane (7%) pour induire l'anesthésie et Isoflurane (5%) pour le maintien de l'anesthésie pour la durée du scan entre 10-15 min (2x / porc); Voie respiratoire - masque nasal;

Analgésique :

Antibiotique :

9. Énumérez les produits utilisés sous contrôle fédéral

NOM	CARACTÉRISTIQUES	GESTION / SUIVI

10. Caractéristiques des produits utilisés ne nécessitant pas l'obtention de permis gouvernementaux :
(Autres que ceux décrits à la question 8)

NOM	VOIE (i.m., s.c., orale, etc.)	DOSE
Stressnill	i.m.	2.2 mg/kg poids vif
Sevoflurane	Respiratoire - masque nasal	7% / L de O2
Isoflurane	Respiratoire - masque nasal	5%/ L de O2

- Y a-t-il des effets secondaires suite à l'apport de ce(s) produit(s)? Oui ☐ Non ☑
- **Si oui, lesquels?**

11. Caractéristiques des produits utilisés nécessitant l'obtention de permis gouvernementaux :
(Autres que ceux décrits à la question 9)

		NOM	VOIE	DOSE
☐	Agents infectieux (ex: bactérie, virus)			
☐	Matériel biologique (ex: vaccin)			
☐	Agent chimique (ex: médicament)			
☐	Radioisotope (ex: I_{125})			
☐	Agents à biorisque			
☐	Autres			

- Est-ce que vous avez l'approbation institutionnelle relative à l'utilisation de ces produits?
- Précisez pour chaque produit :
- Quels sont les effets secondaires ou toxiques suite à l'apport de ces produits?

12. Déclaration:

Je m'engage à ce qu'aucune modification ne soit apportée au nombre d'animaux utilisés ou à tout autre aspect de ce protocole sans consultation et approbation préalables du Comité institutionnel de protection des animaux (CIPA) du Centre de recherche et de développement de Sherbrooke (CRDS).

J'ai lu les sections pertinentes des *Lignes directrices du CCPA: les soins et la gestion des animaux en science* ainsi que les autres directives et politiques du Conseil canadien de protection des animaux (CCPA) et je certifie que tous les animaux utilisés dans le cadre du présent travail seront manipulés et soignés conformément aux principes et règlements énoncés dans les documents et le site web du CCPA à l'adresse suivante: https://www.ccac.ca/fr/index.html.

2023-02-07	Digitally signed by Remus, Aline Date: 2023.02.07 16:17:06 -05'00' Adobe Acrobat Reader version: 2022.003.20310
Date	Signature du responsable du projet

13. <u>Évaluation du Comité institutionnel de protection des animaux :</u>

Approbation (tel que lu à la date inscrite ci-dessous) ☑

Commentaires :

Approbation conditionnelle ☐

Commentaires :

Délais :

Désapprobation ☐

Commentaires :

Appel :

BuenoDalto, Danyel — Digitally signed by BuenoDalto, Danyel Date: 2023.02.07 21:10:29 -05'00'

Signature du mandataire du sous-comité et DATE

Methot, Steve — Signature numérique de Methot, Steve Date : 2023.02.09 15:18:41 -05'00'

(Assurez-vous qu'une boîte-réponse soit cochée à la question 13)

Signature du président du comité et DATE

Steve Méthot

- ☐ Aline Remus
- ☐ Mélissa Duplessis
- ☐ Daniel Ouellet
- ☒ Danyel Bueno Dalto
- ☐ Remplaçant : _____ (Lettres carrées s.v.p.)

RELAÇÕES DE AMINOÁCIDOS EM DIETAS DE SUÍNOS ALIMENTADOS ATRAVÉS DO SISTEMA DE ALIMENTAÇÃO DE PRECISÃO

RESUMO – O sistema de alimentação de precisão (IPF) permite o fornecimento de dietas personalizadas para suínos, considerando a variabilidade entre os indivíduos dentro da população. Alimentar suínos com IPF reduz a excreção de nutrientes e os custos de alimentação sem comprometer o desempenho. Em condições comerciais, os suínos são constantemente desafiados por patógenos que ativam seu sistema imunológico, aumentam a variabilidade das exigências nutricionais e reduzem a eficiência de utilização de nutrientes. Portanto, o uso do IPF durante o desafio imunológico possui o potencial de melhorar a eficiência de utilização de nutrientes. Além disso, durante o desafio imune, as necessidades de Met, Thr e Trp aumentam devido ao seu papel nas funções no sistema imune. A associação do IPF com o aumento das relações de Met, Thr e Trp com a Lys pode melhorar a eficiência de utilização de nutrientes e reduzir os impactos negativos da ativação do sistema imune. Além disso, estudos demonstraram que as relações de AA com Lys que otimizam o desempenho de crescimento de suínos alimentados com IPF diferem daquelas utilizadas na alimentação por fases (GPF). No entanto, ainda não está claro se as maiores relações AA com Lys melhoram o desempenho de toda a população ou apenas de suínos em determinadas categorias. Determinar as relações que otimizam o desempenho de animais de diferentes categorias no IPF é uma estratégia nutricional e alimentar para melhorar a precisão com que os suínos são alimentados. O principal objetivo desta tese foi avaliar o efeito de diferentes relações de AA para Lys em suínos alimentados com IPF. Para isso, foram realizados dois experimentos. O primeiro experimento teve como objetivo avaliar o efeito do IPF e o aumento das relações de Met, Thr e Trp com Lys em suínos sob desafio sanitário. Os resultados demonstraram que o IPF prejudicou o desempenho e não melhorou a eficiência de utilização de nutrientes, e o aumento da relação de AA com Lys não melhorou o desempenho dos suínos sob desafio sanitário. Esses resultados sugerem que (i) ao não considerar o efeito do desafio imunológico nas exigências nutricionais,

o IPF prejudica o desempenho de crescimento; e (ii) os benefícios de melhorar as relações de Met, Thr e Trp com Lys podem depender das relações usadas para formular as dietas, da intensidade do desafio sanitário e do consumo de AA. O segundo experimento teve como objetivo avaliar o efeito de aumentos graduais na relação de Thr com Lys em suínos de diferentes categorias de taxa de crescimento e alimentados com IPF. Os resultados demonstraram que aumentar a relação de Thr com Lys melhorou o desempenho dos suínos com baixa taxa de crescimento, mas não aumentou o GPD das demais categorias. Esses resultados indicam que, no IPF, as relações de AA que otimizam o desempenho são dinâmicas e variam de acordo com a taxa de crescimento dos animais. Esta tese destaca a necessidade de considerar o efeito do sistema imunológico nas exigências dos suínos para melhorar a precisão com a qual o IPF estima as exigências individuais. Além disso, foi mostrado que apesar de o IPF considerar a variabilidade da população para estimar as exigências de Lys, as relações dos demais AA com Lys também devem ser avaliadas ao utilizar o IPF. Portanto, o status do sistema imunológico e as categorias de taxa de crescimento devem ser considerados ao aplicar o IPF. Dessa maneira, a presente tese contribui significativamente para melhorar a aplicabilidade do IPF.

Palavras-chave: alimentação individual, desafio sanitário, eficiência de utilização, exigências nutricionais, categorias de crescimento

DIETARY AMINO ACID RATIOS FOR PIGS FED WITH PRECISION FEEDING SYSTEM

ABSTRACT – The precision feeding system (IPF) allow the provision of tailored diets to pigs accounting for the variability between individuals within the population. Feeding pigs with IPF reduces nutrient excretion and feeding costs without compromising growth performance. In commercial conditions, pigs are constantly challenged with pathogens, which activate the pig's immune system, increase the nutritional requirements variability and reduce nutrient efficiency. Therefore, the use of IPF during the immune challenged may improve nutrient efficiency. Additionally, during the immune challenge, the requirements of Met, Thr and Trp increase due to their role in immune functions. The association of IPF and increased Met, Thr and Trp to Lys ratios may improve the nutrient efficiency and reduce the negative impacts of the immune system activation. Furthermore, it was previously reported that the AA to Lys ratios that optimize the growth performance of pigs fed with IPF differ from those ratios used in the group-phase feeding (GPF). However, it is still not clear if the increased AA to Lys ratios improve the performance of the entire population or only certain pig categories. To determine the ratios that improve the performance of pig categories within IPF is a nutritional and feeding strategy to improve the accuracy with which pigs are fed. The main objective of this thesis was to evaluate the effect of different AA to Lys ratios for pigs fed with IPF. In order to do that, two experiments were carried out. The first experiment aimed to evaluate the effect of IPF and increased Met, Thr and Trp to Lys ratios for sanitary challenged pigs. The results demonstrated that IPF impaired the performance and did not improve nutrient efficiency and the increased AA to Lys ratio did not improve the performance of sanitary challenged pigs. These results suggests that (i) by not accounting for the effect of the immune challenge in the nutritional requirements, the IPF impairs the growth performance; and (ii) the benefits of improving the Met, Thr and Trp to Lys ratios may depend on the ratios used to formulate the diets, the intensity of the sanitary challenge and the AA

intake. The second trial aimed to evaluate the effect of graded increases in Thr to Lys ratios for pigs with different growth rate categories and fed with IPF. The results demonstrated that increasing the Thr to Lys ratio improved the performance of slow growth rate pigs but did not affect the other growth categories. Such outcomes indicate that, within IPF, the AA ratios that optimize the performance are influenced by the growth rate category. This thesis highlights the need of considering the immune system effect on the pigs' requirements to improve the accuracy with which the IPF estimate individual requirements. Additionally, it was shown that even though IPF accounts for the population variability to determine the Lys requirements, the other AA ratios must also be considered when using IPF. Therefore, immune system status and growth rate categories should be considered when applying the IPF. Altogether, the present thesis significantly contribute to improve the applicability of the IPF system.

Key-words: efficiency of utilization, growth categories, individual feeding, nutritional requirements, sanitary challenge

CAPÍTULO 1 – CONSIDERAÇÕES GERAIS

1.1. Introdução

Durante as última decadas, o sistema de produção de suínos se intensificou consideravelmente para suprir a crescente demanda por produtos de origem animal. Um dos principais desafios enfrentados pela indústria suinícola é o de produzir com eficiência e custos mínimos. Além disso, a elevada contribuição da suinocultura na excreção de nitrogênio (N) e fósforo (P) no ambiente tem pressionando produtores e nutricionistas a reavaliar os programas nutricionais e de alimentação utilizados (Garcia-Launay et al., 2014). Convencionalmente, os suínos são criados em grupos e alimentados com uma dieta única durante uma fase do ciclo de produção (GPF - G*roup-Phase Feeding*). Entretanto, mesmo fazendo parte de um grupo, os suínos possuem peso, ganho de peso, consumo e até idades diferentes, resultando na necessidade de diferentes quantidades de nutrientes para cada animal. Dessa forma, o sistema de alimentação por fases desconsidera a variabilidade existente entre animais. As composições das dietas fornecidas se assemelham às exigências dos animais mais exigentes da população (Hauschild et al., 2010; NRC, 2012; Remus et al., 2020). Consequentemente, a maioria dos suínos recebe mais nutrientes do que o requerido durante todo o ciclo de produção (Andretta et al., 2014). O excesso de nutrientes ingerido é excretado e contribui com o aumento dos custos e diminuição da sustentabilidade da cadeia suinícola.

A eficiência de utilização de nutrientes pode ser otimizada através do fornecimento de dietas mais precisas que se adequem às exigências individuais dos suínos. Embora seja possível aumentar o número de fases de alimentação, a variação entre indivíduos não é considerada no GPF. Portanto, este sistema de alimentação impossibilita que suínos sejam alimentados individualmente com dietas ajustadas às suas exigências. O primeiro modelo de alimentação de precisão (IPF – *Individual Precision Feeding*) capaz de estimar exigências individuais de suínos em tempo real foi desenvolvido por Hauschild et al. (2012). O modelo utiliza o histórico de consumo de ração diário (CRD) e peso corporal

(PC) de cada indivíduo para estimar o seu CRD, PC e ganho de peso diário (GPD) futuros. Essa variáveis são então utilizadas em equações fatoriais para determinar a exigência de lisina (Lys) para obtenção do desempenho estimado. Experimentos conduzidos com o IPF demonstraram que sua utilização reduz 27% do consumo de Lys e 30% da excreção de N (Andretta et al., 2014). Ademais, também foi observado redução de 14% da excreção de P e de 10% dos custos com a alimentação (Pomar et al., 2014; Andretta et al., 2016). Assim, o IPF se configura como uma ferramenta eficaz para aumentar a eficiência de utilização de nutrientes, lucratividade e sustentabilidade do sistema de produção de suínos.

Apesar de vários estudos terem sido conduzidos para avaliar o IPF (Andretta et al., 2014; Cloutier et al., 2015; Andretta et al., 2016; Remus et al., 2019), nenhum deles avaliou sua utilização para suínos imunologicamente desafiados. O desafio imune resulta na liberação de citocinas que reduzem o anabolismo e aumentam o catabolismo do músculo esquelético e tecido adiposo (Johson et al., 1997; Cooney et al., 1997). Além disso, nutrientes que seriam utilizados para crescimento e mantença são redirecionados para o suporte da resposta imunológica (Le Floc'h et al., 2018). Esses fatores resultam no aumento da exigência de mantença e na redução do crescimento dos suínos (Huntley et al., 2018). As respostas individuais frente ao desafio aumentam a variabilidade das exigências e reduzem a eficiência de utilização de nutrientes (Huntley et al., 2018, Ortiz et al., 2024). Portanto, a aplicação do IPF para esses animais poderia melhorar sua retenção de nutrientes por fornecer dietas ajustadas às suas exigêcias individuais.

Neste cenário, duas preocupações são levantas acerca da utilização do IPF. A primeira reside no fato dele não considerar as alterações metabólicas decorrentes da resposta imune no cálculo das exigências nutricionais. A segunda é o fato de que o baixo CRD e menor GPD durante o desafio são utilizados no histórico do animal para determinar seu desempenho futuro. Após a resolução da resposta imune, os animais tendem a recuperar o CRD e GPD (Sandberg et al., 2016). Dessa forma, o CRD e GPD que são estimados utilizando os dados obtidos durante o desafio podem subestimar o desempenho futuro e resultar no

fornecimento de dietas com menor concentração nutricional do que aquela requerida pelos animais.

Adicionalmente, estudos demonstraram que a ativação do sistema imunológico aumenta as relações de AA com Lys de determinados AA que possuem papel importante no sistema imune. Esses AA são comumente definidos como AA funcionais. Dos AA funcionais utilizados, a treonina (Thr), metionina (Met) e triptofano (Trp) atenuam os efeitos da ativação imunológica no desempenho e retenção de nutrientes (Rodrigues et al., 2021; Valini et al., 2023). Seu benefício está associado ao aumento na proteção à translocação de patógenos do lúmen intestinal para a corrente sanguínea, proteção antioxidante e redução da ativação imune crônica (Le Floc'h et al., 2018). Assim, o aumento das relações de AA funcionais associados ao IPF se configuram com uma possível estratégia nutricional e alimentar para suínos submetidos a um desafio imune.

Além da utilização do IPF para animais em desafio imunológico, questionamentos são levantados acerca da aplicação das relações AA:Lys do GPF no IPF. Estudos demonstraram o aumento linear no ganho de peso e deposição de proteína em suínos alimentados com relações crescentes de Met e Thr:Lys no IPF (Remus et al., 2019a, b). Além disso, o coeficiente de variação no ganho de peso diário diminuiu nas maiores relações de Met:Lys, sugerindo que o beneficio do aumento das relações foi mais expressivo em animais de uma determinada categoria da população. Isso sugere que o aumento das relações AA:Lys no IPF mehora a expressão do potencial de crescimento de suínos. No entanto, a resposta de suínos de diferentes categorias e alimentados com diferentes relações AA:Lys ainda não foi elucidada.

Sob essa perspectiva, ao considerar o aumento da variabilidade das exigências nutricionais entre os indivíduos de uma população de suínos desafiados, a associação do IPF com a suplementação de AA funcionais para suínos em condições de desafio imunológico possui o potencial de amenizar a queda de desempenho e melhorar o aproveitamento de nutrientes dos mesmos. Adicionalmente, a determinação das relações AA:Lys que otimizam o desempenho de suínos de diferentes categorias de crescimento no IPF pode

maximizar o crescimento da população e diminuir a variabilidade entre indivíduos. Em suma, o fornecimento de relações AA:Lys no IPF acima daquelas utilizadas no GPF podem aumentar o desempenho e a utilização de nutrientes, reduzindo a poluição ambiental e contribuindo para a lucratividade e sustentabilidade do sistema de produção suinícola.

1.2. Revisão de Literatura

1.2.1. Limitações dos métodos empírico e fatorial na determinação das exigências nutricionais de suínos

O aumento da eficiência de produção com menor custo e maior sustentabilidade estão entre os principais desafios enfrentados por nutricionistas de suínos. Apesar de as dietas formuladas vizarem maximizar o desempenho, a eficiência de utilização (eficiência com a qual os suínos convertem proteína dietética em muscular) geralmente é baixa. Flachowsky and Kamphues (2012) e Dourmand et al. (1999) demonstraram que a eficiência de utilização de N varia de 15% a 33%. Essa ineficiênca na retenção é oriunda das perdas endógenas relacionadas a descamação e secreções do trato gastrointestinal, da disponibilidade para absorção de nutrientes de diferentes ingredientes e do excesso de AA fornecidos nas dietas. Neste contexto, se torna difícil manipular a eficiência de utilização através da modificação da utilização dos AA em atividades metabolicas devido à sua complexidade e essencialidade. Dessa maneira, a forma mais eficaz de se aumentar a eficiência de utilização de nutrientes é a sua manipulação dietética com o fornecimento de dietas com composição mais próxima às exigências nutricionais dos animais. Portanto, a correta determinação das exigências nutricionais se faz necessária para aumentar a eficiência de utilização de nutrientes.

Programas de alimentação modernos utilizam o fornecimento de uma dieta única para grupos de suínos durante uma fase do ciclo de produção (Figura 1.1). As exigências nutricionais utilizadas durante a formulação são geralmente obtidas através da utilização do método empírico ou fatorial. No método empírico, as exigências são definidas como a menor quantidade de um nutriente que deve ser

fornecido para maximizar uma resposta da população durante um intervalo de tempo (Hauschild et al., 2010). Por outro lado, o método fatorial determina a exigência de um nutriente em um período específico através da soma das exigências de mantença e produção (crescimento, produção de leite) utilizando o indivíduo médio como referência (Hauschild et al., 2010). Embora amplamente utilizados, ambos possuem limitações. Os modelos assumem que a resposta da população ou do indivíduo a um determinado nível do nutriente é estática. Ademais, a estimativa do método empírico é dependente da quantidade de níveis, do modelo utilizado na determinação da exigência (poinomial quadrático, linear-plateau, quadrático linear-plateau) e do tempo de duração do estudo. Além disso, o método fatorial geralmente assume que o animal médio é o animal que melhor representa a população. Entretanto, estudos demonstraram que o ganho de peso diário dos suínos é maximizado quando estes são alimentados com dietas cuja concentração é igual ou superior à exigência do animal presente no percentil 80 (Hauschild et al., 2010). Portanto, a utilização do animal médio resulta em valores de exigência inferiores àqueles necessários para maximizar a resposa da população (Pomar et al., 2003; Brossard et al., 2009; Hauschild et al., 2010). Finalmente, as condições experimentais onde as exigências foram determinadas muitas vezes se diferem daquelas observadas em outras granjas. Dessa maneira a exigência estimada na população referência pode ser diferente das demais populações.

Além das limitações conceituais, o método empírico e fatorial falham ao não considerarem a variabilidade das exigências nutricionais existentes na população. Cada indivíduo de uma população de suínos possui consumo, peso e ganho de peso diferente dos demais. Portanto, suas exigências evoluem em um padrão diferente ao longo do tempo em uma variação denominada tempo-dependente (Figura 1.1; Pomar e Remus, 2019). Ademais, mesmo quando situados no mesmo estágio do ciclo produtivo, suínos possuem diferentes taxas de crescimento, consumo e peso corporal. Assim, além da variação tempo-dependente, a variação entre indivíduos faz com que cada animal de uma população tenha uma exigência diária diferente dos demais. Portanto, essas duas

variações, tempo-dependente e entre individuos, dificultam o fornecimento de dietas ajustadas às exigências dos suínos.

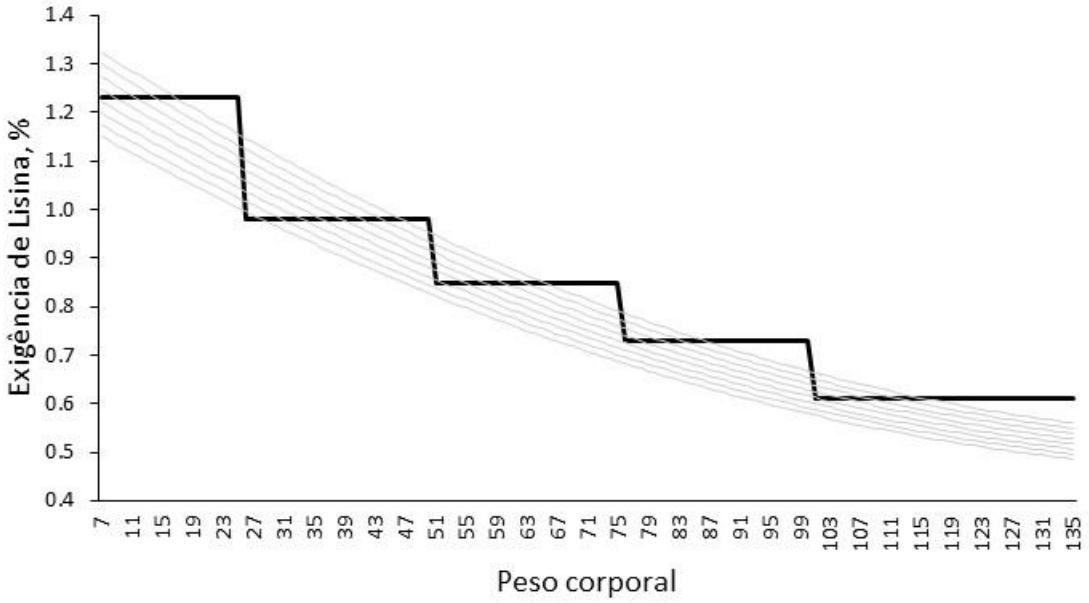

Figura 1.1. Representação gráfica das variações das exigências de suínos (cinza), e concentração dietética das dietas fornecidas em um sistema de alimentação convencional com 5 fases (preto) sugerido pelo NRC (2012). As linhas cinzas demonstram a variação tempo-dependente e entre indivíduos existentes no sistema de produção de suínos.

Além disso, no GPF, as concentrações dietéticas estimadas para cada fase de alimentação se assemelham às exigências dos animais mais exigentes (Hauschild et al., 2010; Remus et al., 2020). Dessa forma, alguns suínos são sub-alimentados enquanto a maioria dos indivíduos é superalimentada ao longo de todo ciclo produtivo (Andretta et al., 2014). Suínos sub-alimentados deverão ingerir uma maior quantidade de ração para atender suas exigências nutricionais e prevenir redução do seu desempenho. Por outro lado, a superalimentação não resulta em uma melhora significativa do desempenho (Pomar et al., 2003). Como resultado, o excesso de nutrientes ingerido é excretado diminuindo sua eficiência de utilização e aumentando os gastos com alimentação. Neste cenário, as limitações do GPD inviabilizam a utilização deste sistema no fornecimento de dietas com concentrações nutricionais mais próximas às exigências individuas

dos suínos. Ferramentas emergentes como o IPF se mostram eficazes por permitir o fornecimento de dietas que considerem as variações tempo-dependentes e entre indivíduos na estimativa das exigências nutricionais.

1.2.2. Implementação do sistema de alimentação de precisão

A alimentação de precisão pode ser definida como a aplicação de técnicas de alimentação que permitem o fornecimento de uma dieta ajustada às exigências nutricionais dos animais considerando a variação tempo-dependente e entre indivíduos (Pomar e Remus, 2019). Para a sua aplicação, se fazem necessários (i) a avaliação precisa da composição dos ingredientes; (ii) a determinação correta das exigências nutricionais individuais; (iii) a formulação de dietas balanceadas que atendam as exigências com o menor excesso possível de nutrientes; e (iv) o ajuste diário do fornecimento de ração para que a concentração e composição dietética atendam a exigências nutricional de cada individuo (Pomar et al., 2009).

O primeiro modelo matemático capaz de estimar exigências individuais diárias considerando as variações entre indivíduos foi desenvolvido por Hauschild et al. (2012). O modelo possui um componente empírico e um mecanicista (Figura 1.2). O componente empírico utiliza o histórico de CRD e PC para estimar o CRD, PC e GPD do dia seguinte de cada indivíduo. Portanto, este modelo difere-se dos determinísticos usualmente utilizados no cálculo das exigências nutricionais por considerar a variação entre indivíduos. Por sua vez, o componente mecanicista utiliza essas estimativas como *input* em equações fatoriais para determinar as exigências diárias para mantença e crescimento, em gramas de Lys, necessárias para que o animal desempenhe conforme o estimado.

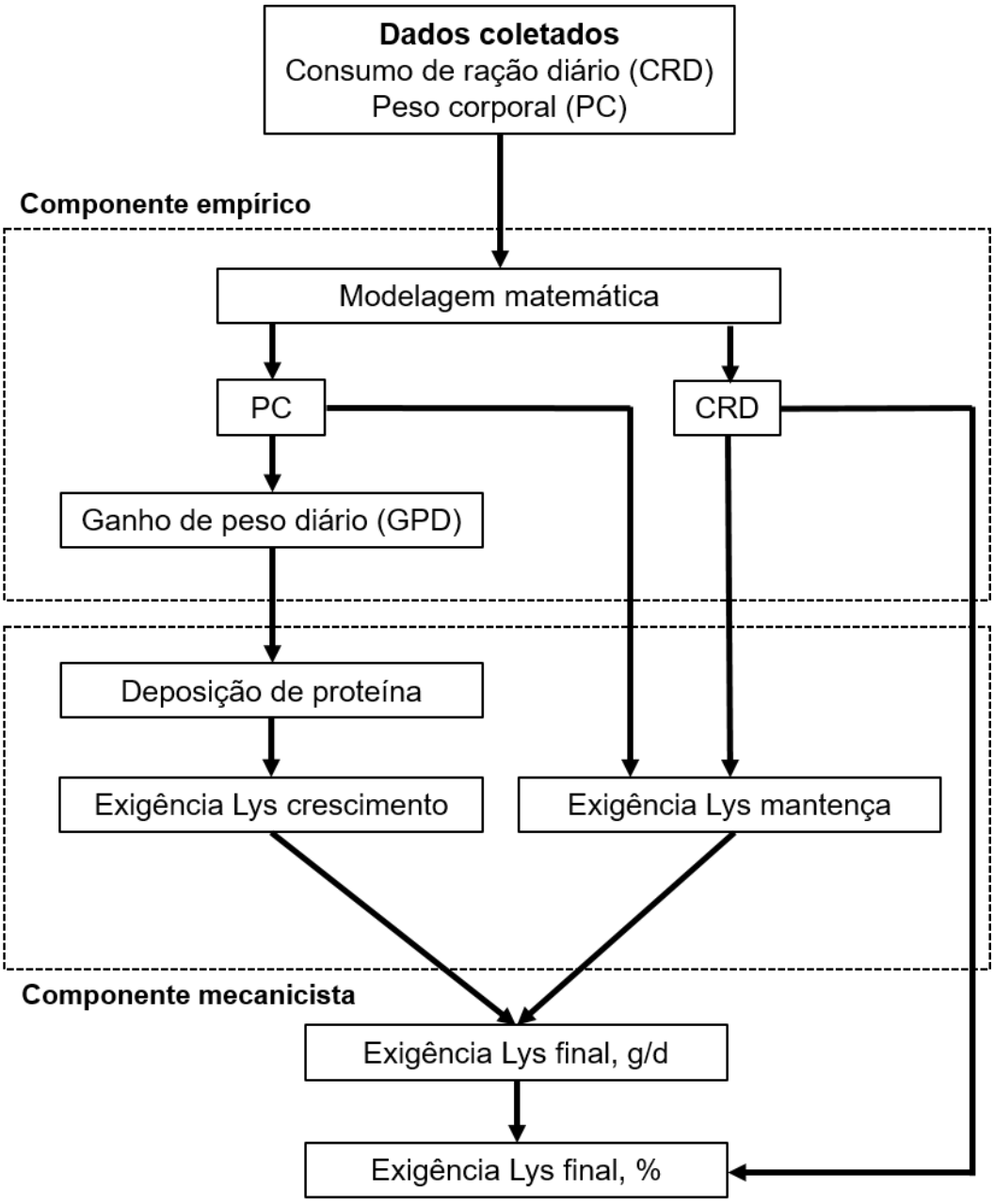

Figura 1.2. Descrição geral do modelo desenvolvido por Hauschild et al. (2012). O modelo possui um componente empírico e um mecanicista utilizados para estimar a exigência individual diária de cada animal de acordo com o padrão individual de crescimento e consumo.

As exigências em Lys para mantença são estimadas através da soma das perdas endógenas basais (0.313 g Lys/kg MS × CRD) com as perdas relacionadas à descamação do trato gastro-intestinal (0.0045 g Lys/kg $PC^{0.75}$ × $PC^{0.75}$) e com as perdas de Lys relacionadas ao *turn over* proteico (0.0239 g Lys/ kg $PC^{0.75}$ ×

$PC^{0.75}$; van Milgen et al., 2008). As exigências de Lys ileal standardizada para crescimento são estimadas assumindo que 16% do GPD é composto por proteína (de Lange et al., 2003) e que 7% dessa proteína é Lys (Mahan e Shields, 1998). Após a determinação da exigência de Lys para crescimento, é considerada uma eficiência de utilização da Lys de 72% (Möhn et al., 2000). Posteriormente, a exigência diária (soma de mantença e crescimento) estimada em gramas é dividida pelo CRD (g) estimado para que seja calculado a concentração de Lys em porcentagem da dieta a ser fornecida no dia seguinte.

1.2.3. Obtenção de dados e fornecimento de dietas individuais

A exigência nutricional dos suínos se altera continuamente. Portanto, se faz necessária a utilização de equipamentos que possibilitem a obtenção automatizada e o processamento de dados para adequada determinação das exigências individuais e ajustes das dietas fornecidas. Os dados necessários para a aplicação do modelo de Hauschild et al. (2012) são o PC e CRD individual. O PC individual pode ser mensurado com a utilização de balanças e pesagens individuais. Porém, a obtenção do CRD individual é dificultada devido ao alojamento dos animais em grupo. Portanto, estações de alimentação automáticas são utilizadas para obtenção de valores de consumo individual (Figura 1.3).

Brincos eletrônicos com número de identificação únicos são fixados às orelhas dos suínos e os possibilitam acessar os alimentadores. Os comedouros possuem capacidade de armazenar até quatro rações distintas em seu interior e são construídos para misturar até quatro dietas em diferentes proporções em uma única refeição. Ao acessarem as estações de alimentação, os suínos requisitam o alimento ao acionarem um dispositivo no interior do alimentador. Uma vez requisitado, parafusos de Arquimedes fornecem quantidades volumétricas de ração (Pomar et al., 2011). A quantidade de ração fornecida por requisição (de 15 a 25 gramas) e o tempo mínimo permitido entre requisições podem ser ajustados para garantir que os suínos consumam todo o alimento fornecido evitando desperdícios. Isso garante a precisão do consumo de ração registrado em cada

refeição. Após o fornecimento da ração, o software do alimentador registra a quantidade de ração fornecida e assume que esta mesma quantidade foi ingerida pelo animal. Após a obtenção e processamento dos dados, as exigências para cada indivíduo são estimadas para o dia seguinte utilizando o modelo de Hauschild et al. (2012).

Para atender as diferentes exigências nutricionais dos animais de uma população, duas dietas são misturadas. As dietas são comumente denominadas de dietas A e B. A dieta A (alta densidade de nutrientes) é formulada para atender as exigências nutricionais do animal mais exigente no início da fase de crescimento. Por outro lado, a dieta B (baixa densidade de nutrientes) é formulada para atender as exigências do animal menos exigente no final da fase de terminação. Os demais AA e nutrientes são fornecidos em uma porporção constante com a Lys. Dessa maneira, as misturas das dietas A e B são capazes de atender qualquer exigência nutricional entre o início do crescimento e o final da terminação.

Portanto, após determinação das exigências individuais, a proporção das misturas entre as dietas A e B são carregadas no sistema dos alimentadores automáticos. Conforme o animal requisita o alimento no dia seguinte, os alimentadores entregam quantidades volumétricas de ração com a proporção entre A e B designada para cada animal. Assim, é possível alimentar suínos individualmente com diferentes dietas mesmo quando estes estão alojados em grupo. A densidade das dietas deve ser mensurada semanalmente para calibração dos comedouros e garantir o correto fornecimento das quantidades estipuladas por requisição.

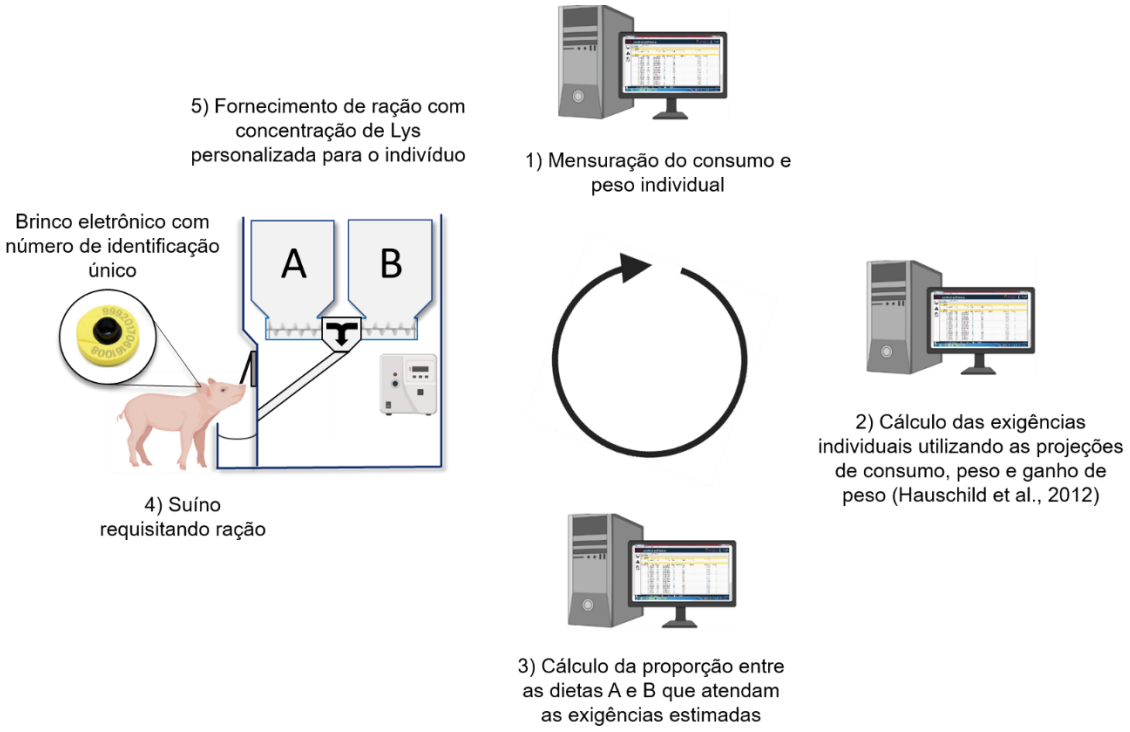

Figura 1.3. Esquema da integração entre as estações de alimentação automáticas e o modelo de alimentação de precisão desenvolvido por Hauschild et al. (2012). O consumo e o peso dos animais são mensurados e utilizados na predição do consumo, peso e ganho de peso futuros (1). Os parâmetros estimados são utilizados para determinar as exigências individuais de Lys (2). Posteriormente, a proporção entre as dietas A e B é calculada e inserida no sistema para que cada refeição fornecida ao animal contenha a concentração de Lys calculada (3). Ao requisitar a ração no alimentador (4), o suíno recebe refeições com proporção pré-estabelecida entre as dietas A e B (5).

1.2.4. Benefícios e perspectivas da utilização do sistema de alimentação de precisão

Em um estudo realizado por Pomar et al. (2014), os autores forneceram dietas ajustadas diariamente de acordo com a exigência média da população. Eles observaram que considerar a variação tempo dependente da população resultou na redução de 7% do consumo de proteína e 12% da excreção de N. Portanto, modelos como o de Hauschild et al. (2012) que consideram as variabilidade tempo-dependente e entre indivíduos possuem elevado potencial de redução de consumo e excreção de nutrientes. O modelo de Hauschild et al. (2012) foi calibrado em dois estudos (Zhang et al., 2012; Cloutier et al., 2015) e validado por

Andretta et al. (2014 e 2016). Estes demonstraram que o IPF reduz 27% do consumo de Lys e 30% da excreção de N. Ademais, os autores observaram redução de 14% da excreção de P. Embora os custos com a alimentação dependam do valor de *commodities* e de custos associados à logística de obtenção das mesmas, Pomar et al. (2014) evidenciaram em uma simulação que o IPF pode reduzir até 10% dos custos com a alimentação. É importante salientar que não houve diferença no consumo e eficiência alimentar dos suínos nos estudos que contrastaram o IPF com o GPF (Andretta et al., 2014 e 2016; Santos et al., 2018). Esses resultados demonstram que os ganhos atrelados a utilização do IPF são decorrentes apenas da modificação da composição da dieta fornecida e não da quantidade de ração ingerida pelos suínos. Assim, o IPF se configura como uma ferramenta eficaz para aumentar a eficiência de utilização de nutrientes, a lucratividade e a sustentabilidade do sistema de produção de suínos.

O principal objetivo da realização de experimentos avaliando o IPF é o seu aperfeiçoamento para que ele possa ser utilizado em granjas comerciais. Contudo, a maioria dos experimentos que avaliaram o IPF foram realizados com animais hígidos em condições experimentais (Andretta et al., 2014; Cloutier et al., 2015; Remus et al., 2019). Em granjas comerciais, suínos são frequentemente expostos a patógenos que ativam o seu sistema imunológico. Devido a respostas individuais dos hospedeiros, o desafio aumenta a variabilidade das exigências nutricionais (Figura 1.4; Ortiz et al., 2024) e diminui a eficiência de utilização de nutrientes (Huntley et al., 2018).

Sob essa perspectiva, se faz interessante a avaliação do IPF para suínos desafiados sob a perspectiva de que esse sistema de alimentação pode melhorar a eficiência de utilização de nutrientes desses animais. As alterações metabólicas decorrentes da ativação imunológica e a utilização do IPF para suínos desafiados serão discutidos de forma mais aprofundada nos tópicos seguintes.

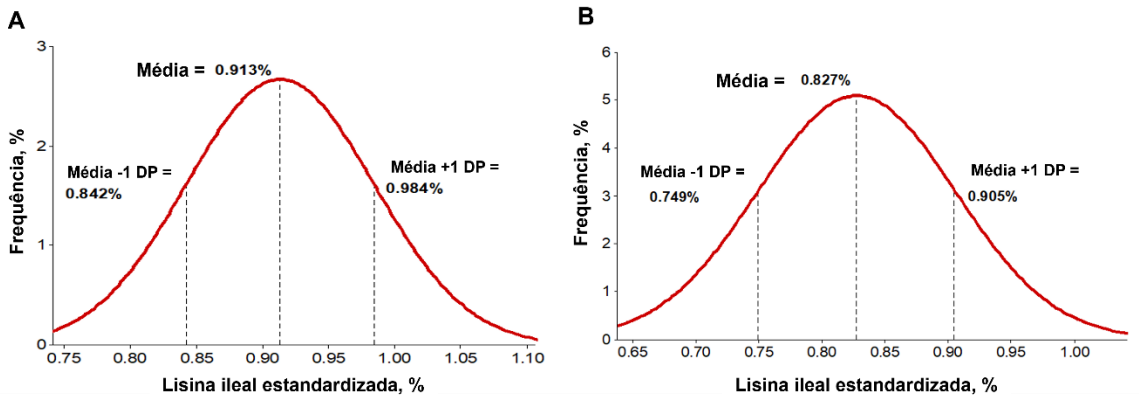

Figura 1.4. Histograma das exigências individuas de lisina de suínos alojados em condições sanitárias boas (A) e condições sanitárias ruins (B) caracterizada por inoculação oral com *Salmonella* Typhimurium e ambiente contaminado com fezes de suínos. Os gráficos apresentam a média ± 1 desvio padrão (DP) das exigências de lisina. Adaptado de Ortiz et al. (2024).

1.2.5. Efeito da ativação do sistema imune no metabolismo e desempenho de suínos

Em condições comerciais, suínos são expostos a diferentes tipos de estressores inerentes ao sistema de produção. Por exemplo, estressores ambientais (densidade de alojamento) ou sociais (mistura de lotes) que dificultam a higienização das intalações e aumentam a pressão patogênica. Consequentemente, suínos entram em contato com microrganismos carreados pelo ar, água, alimentos e fezes de outros animais. Embora os animais possuam barreiras imunes (pele, tosse, espirro, suco gástrico), alguns microrganismos são capazes de superar essas defesas. Ao romper as barreiras imunológicas, patógenos se translocam para a corrente sanguínea e iniciam um quadro de sépticemia (infecção generalizada). Como consequência, células do sistema imune sintetizam citocinas pró-inflamatórias (IL-6, IL-1β e TNF-α) que iniciam uma cascata de eventos e modificam o metabolismo dos suínos. Um dos efeitos mais observados da ativação imune é a anorexia, responsável por reduzir o aporte de nutrientes que poderiam ser utilizados para deposição muscular e crescimento (Johnson, 1998). No entanto, as mudanças metabólicas (aumento da temperatura corporal e síntese de componentes do sistema imune) aumentam a exigências de mantença e reservas corporais devem ser mobilizadas para suprir a demanda

nutricional. Em um experimento avaliando o efeito de injeções intramusculares de lipopolissacarídeos (LPS), Huntley et al. (2018) observaram que a resposta febril de suínos aumentou a exigência energética de mantença em 23,3%.

O tecido adiposo é a principal reserva energética corporal e ácidos graxos são mobilizados em cenários de demanda energética. O aumento da concentração de citocinas pró-inflamatórias está associado a maior resistência a insulina no tecido adiposo, diminuindo a síntese de ácidos graxos (Hotamisligil et al., 1993), consequentemente, as reservas energéticas corporais. Além disso, a resposta imune reduz a captação de ácidos graxos livres do plasma para o interior dos adipócitos devido a redução da ação da enzima lipase lipoproteica (Johnson et al., 1997). Finalmente, a sinalização do TNF-α em receptores de membrana dos adipócitos ativa o fator de transcrição nuclear kapa beta (NF-κB). Por sua vez, o NF-κB ativa a enzima lipase hormônio sensível, resultando na liberação de ácidos graxos das reservas de lipídios nos adipócitos (Grant e Stephens, 2015). Portanto, a ativação imunológica está associada ao aumento da concentração sérica de triglicerídeos e menor deposição de lipídios. Huntley et al. (2018) observaram que injeções de LPS diminuiram 23% da retenção de energia na forma de lipídios. Valini et al. (2023) observaram redução de 26% na deposição de lipídios diária de suínos em crescimento desafiados por *Salmonella* Typhimurium e alojados em um ambiente com condições ruins de higiene.

Além das alterações no metabolismo de lipídios, AA também são mobilizados devido a dois processos simultâneos: diminuição da síntese e aumento do catabolismo proteico (Johnson, 1997). A redução da síntese é ocasionada pela diminuição do processo de iniciação da tradução de mRNA e à diminuição da concentração sérica de hormônios anabolizantes como o fator de crescimento semelhante a insulina 1 (IGF-1) (Cooney et al., 1997). Por outro lado, o catabolismo ocorre devido a ativação de lisossomos e do complexo ubiquitina-proteassoma e maior atividade das calpaínas (Cooney et al., 1997). Os AA oriundos da proteólise muscular são prioritariamente utilizados pelo fígado para síntese de componentes do sistema imune e como substrato para gliconeogênese (Cooney et al., 1997; Johnson, 1997; Le Floc'h et al., 2004). Além disso, uma

maior proporção de AA deve ser mobilizada do músculo devido as diferenças entre os perfis de AA muscular e o requerido para a resposta imune (Reeds et al., 1994). Alterações no *turn over* do tecido muscular esquelético interferem diretamente nas taxas de deposição muscular e no balanço de N (De Ridder et al., 2012; Valini et al., 2023). Barcellos et al. (2021) demonstraram que injeções de LPS diminuem 41% da retenção de N. De forma similar, Valini et al. (2023) observaram redução de 8% na eficiência de utilização de N de suínos em crescimento desafiados.

Dessa forma, as modificiações metabólicas oriundas da ativação imune induzem o catabolismo das reservas corporais, reduzem o crescimento e a eficiência de utilização de nutrientes de suínos. Essas alterações contribuem para diminuição da lucratividade e sustentabilidade da cadeia suinícola. Assim, pesquisadores e nutricionistas têm buscado soluções que possibilitem amenizar os efeitos negativos da ativação imunológica. Uma ferramenta que pode ser utilizada é a alimentação de precisão.

1.2.6. Sistema de alimentação de precisão para suínos em desafio imunológico

A intensidade e duração das respostas imunológicas dependem da virulência do patógeno, sua concentração e da sua interação com o hospedeiro (Sandberg et al., 2006; 2007). Consequentemente, reduções no consumo de ração e ganho de peso variam mesmo quando os animais são expostos ao mesmo patógeno (Sandberg et al., 2006). Por considerar que há variabilidade nas respostas imunes dos indivíduos, e consequentemente, do impacto do desafio sobre as exigências nutricionais, o IPF é uma ferramenta interessante a ser utilizada durante o desafio imune.

No entanto, dois questionamentos são levantados a cerca da utilização do IPF para estimar as exigências de suínos imunologicamente desafiados. O primeiro reside no fato de que as equações fatoriais utilizadas para estimar as exigências de Lys foram validadas para animais hígidos. Por não considerar as modificações das exigências nutricionais decorrentes da ativação imunológica (aumento da mantença, por exemplo), as estimativas do IPF podem subestimar

as exigências. O segundo está relacionado com o efeito da redução de desempenho durante o desafio sobre estimativas das exigências subsequentes. Sandberg et al. (2006) e Pastorelli et al. (2012) demonstraram que o desempenho de animais desafiados é caracterizado por três fases (Figura 1.5): (i) redução do crescimento em relação aos animais hígidos; (ii) permanência dessa redução durante a ativação do sistema imune; e (iii) recuperação quando os patógenos são eliminados. Durante a recuperação, o metabolismo retorna às condições de homeostase e diminui o desvio de nutrientes para suporte do sistema imune. Isso possibilita a melhora do desempenho dos suínos após o desafio (Sandberg et al., 2006; Pastorelli et al., 2012). O IPF foi desenvolvido para utilizar o histórico de crescimento dos animais para estimar o seu desempenho futuro e fornecer uma dieta que suporte o desempenho estimado. Dessa maneira, os dados de PC e CRD do período de ativação imunológica também são utilizados na predição do PC, GPD e CRD futuros. A utilização desses dados, no entanto, pode subestimar as estimativas de desempenho do modelo, uma vez que o desempenho do animal é comprometido com a ativação da resposta imune. Consequentemente, a quantidade de Lys fornecida pode não atender as exigências dos animais durante o período de recuperação e limitar seu crescimento após o desafio.

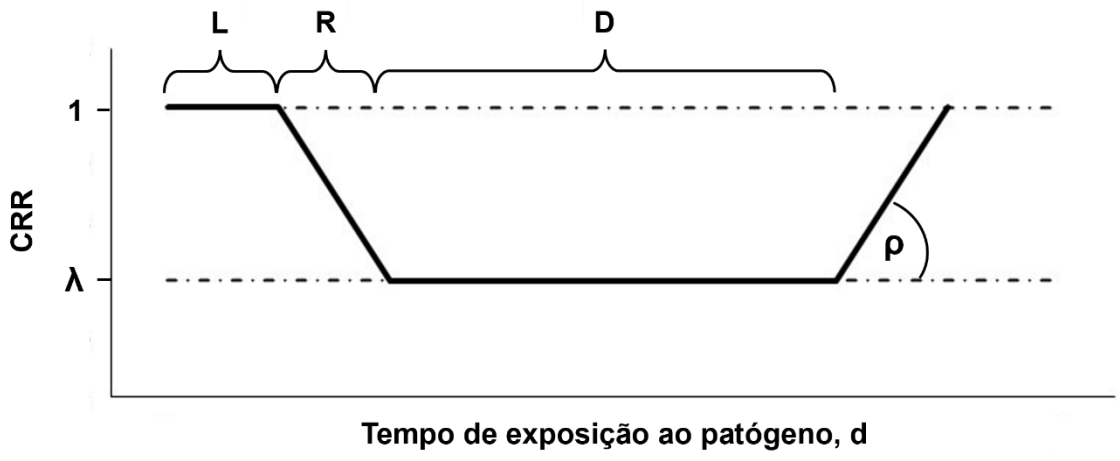

Figura 1.5. Representação gráfica do consumo de ração relativo (CRR) de suínos desafiados. O consumo relativo é representado por 5 parâmetros: tempo entre a exposição e o início da redução (L), tempo de redução (R), redução em relação

ao consumo esperado (λ), duração da redução (D) e taxa de recuperação (ρ). Adaptado de Sandberg et al. (2006).

Portanto, se torna interessante avaliar o efeito da utilização do IPF para suinos submetidos ao desafio sanitário. O estudo dessa estratégia alimentar tem o potencial de melhorar a eficiência de utilização de nutrientes de suínos desafiados.

1.2.7. Suplementação de aminoácidos funcionais para suínos em desafio imunológico

As alterações do metabolismo de AA que ocorrem durante a ativação do sistema imune possuem a finalidade de aumentar a quantidade de AA disponíveis para a construção da resposta imunológica. A maior demanda por AA específicos resulta em modificações nas exigências dos mesmos (Le Floc'h et al., 2004). Dentre eles, destacam-se a Metionina (Met), Treonina (Thr) e o Triptofano (Trp). Esses AA são usualmente denominados como funcionais por atuarem como componentes estruturais de moléculas que auxiliam na resposta imune dos suínos.

Respostas inflamatórias induzem, também, o aumento na produção de espécies reativas de oxigênio (EROs), moléculas que são produzidas pelas mitocôndrias durante o processo de respiração celular. Moléculas de EROs se propagam pelos tecidos corporais e causam a desestruturação de moléculas orgânicas importantes para o funcionamento celular como proteínas, lipídios de membrana e DNA (Mittal et al., 2014). Assim, EROs são utilizadas por macrófagos para que eles exerçam sua ação citotóxica contra microrganismos invasores (Mittal et al., 2014). Entretanto, a estimulação do sistema imune pode resultar na produção exacerbada de EROs, excedendo a capacidade antioxidante do organismo e danificando tecidos corporais (Li et al., 2007; Rémond et al., 2011). Um dos antioxidantes endógenos capazes de neutralizar EROs é a glutationa, molécula sintetizada a partir da cisteína. Uma vez que a Met pode ser convertida em cisteína através do processo de transulfuração, a suplementação de Met

auxilia na manutenção do status redox e integridade celular de suínos (Li et al., 2014).

Dentre os aminoácidos absorvidos no intestino delgado, a Thr é o AA mais retido no sistema porta-hepático com taxas de retenção variando de 60% a 80% (Schaart et al., 2005). Essa maior retenção se deve ao fato de a Thr ser uma importante constituinte da mucina, uma glicoproteína presente no muco que reveste a mucosa intestinal (Lien et al., 1997). Ao atuar como barreira mecânica, o muco impede a adesão de microrganismos na mucosa intestinal e protege os enterócitos contra a ação de microrganismos patogênicos. Além disso, a Thr é o AA mais abundante nas imunoglobulinas (Smith and Greene). Dessa forma, a Thr se configura como um importante componente de defesa imunológica não específico do organismo. Estudos têm demonstrado que a ativação do sistema imune induz o aumento da produção de muco, o que contribui para o aumento da exigência de Thr (Rémond et al., 2009). Finalmente, a Thr é o AA mais abundante nas imunoglobulinas (Smith and Greene, 1947), demonstrando a essencialidade da Thr na resposta imune.

O desafio imune aumenta o catabolismo de Trp em quinurenina pela ação da enzima indoleamina 2,3-dioxigenase produzida por macrófagos e células dendríticas (Popov e Schultze, 2008; Le Floc'h et al., 2011). A quinurenina inibe proliferação de linfócitos T, prevenindo os efeitos deletérios de uma ativação imune crônica (Le Floc'h et al., 2018). Além disso, o Trp atua como precursor da serotonina, neurotransmissor associado ao apetite em suínos (Le Floc'h et al., 2008). Finalmente, o estresse oxidativo ocasionado pela elevada produção de EROs também aumenta a conversão de Trp em quinurenina, sugerindo que o Trp também auxilia na depleção de EROs (Le Floc'h 2004, 2018).

Por desempenharem funções importantes no sistema imunológico, autores avaliaram o efeito da suplementação conjunta de Thr, Met e Trp acima da sua exigência para suínos desafiados. Van der Meer et al. (2016), demonstraram que a suplementação desses AA melhorou o GPD e eficiência alimentar de suínos durante as fases de crescimento e terminação quando estes foram alojados em condições sanitárias ruins. Rodrigues et al (2021) observaram aumento do status

antioxidante de leitões desafiados com inoculação oral de *Salmonella* Typhimurium e alimentados com Thr, Met e Trp. Similarmente, Valini et al. (2023) demonstraram que a suplementação aminoacídica aumentou a deposição de proteína e a eficiência de utilização de N de suínos em crescimento desafiados com inoculação oral de *Salmonella* Typhimurium. Sendo assim, a utilização conjunta do IPF com a suplementação de AA funcionais são estratégias nutricionais e alimentares capazes de aumentar a eficiência de utilização de nutrientes e diminuir os efeitos negativos oriundos do desafio imunológico em suínos.

1.2.8. Relações de aminoácidos na alimentação de precisão

Todos os experimentos realizados com o IPF formularam dietas utilizando o conceito de proteína ideal aplicado no GPF (Andretta et al., 2014,2016; Remus et al., 2019a). A proteína ideal é definida como a composição de aminoácidos em uma dieta que atende perfeitamente às necessidades dos animais para otimizar o crescimento e a produção, minimizando o excesso e a deficiência de nutrientes essenciais (Noblet et al., 1994). No entanto, estudos observaram uma resposta linear na deposição de proteína em suínos alimentados com IPF e relações de Thr e Met com Lys acima das relações que otimizam o desempenho de suínos no GPF (Remus et al., 2019a; Remus et al., 2019b). Além disso, em suínos em fase inicial de crescimento, o aumento na relação Thr com Lys aumento os níveis plasmáticos de albumina, proteína total e proteína C-reativa (Remus et al., 2019c). Esses resultados sugerem que alterações no perfil de aminoácidos podem não apenas melhorar o desempenho de crescimento, mas também potencialmente aumentar a resposta imunológica dos suínos.

Ainda assim, é frequentemente observada uma grande variabilidade na resposta às mesmas proporções de AA dentro dos tratamentos em estudos dose-resposta (Barea et al., 2009; Remus et al., 2019b, c; Soto et al., 2019). As razões para a variabilidade no crescimento ainda são pouco compreendidas. No entanto, estudos demonstraram que o uso de uma única dieta não maximiza o desempenho populacional, pois a composição dietética que otimiza o crescimento

varia entre os indivíduos (Aymerich et al., 2020). Portanto, é possível que diferenças na taxa de crescimento estejam relacionadas a diferenças nas exigências de AA entre os suínos.

Portanto, é crucial investigar o efeito das relações de aminoácidos com lisina em suínos com diferentes categorias de crescimento no IPF. A variabilidade observada nas respostas aos diferentes perfis de aminoácidos destaca a importância de uma abordagem mais detalhada que considere as necessidades específicas de cada categoria de crescimento. Compreender como essas relações impactam cada grupo de suínos pode oferecer insights valiosos para otimizar a formulação das dietas, melhorar o desempenho e promover a eficiência na produção. Isso não só potencializa os benefícios econômicos e produtivos, mas também contribui para o desenvolvimento de sistemas de alimentação mais sustentáveis e adaptados às necessidades individuais dos suínos.

1.2.9. Hipótese

Diante do exposto, foi hipotetizado que a alimentação de precisão, quando associada ao aumento das relações de Met, Thr e Trp com Lys, pode melhorar a eficiência na utilização de nutrientes e reduzir os efeitos negativos da ativação do sistema imunológico. Além disso, sugere-se que as relações ótimas de Thr com Lys na alimentação de precisão podem variar conforme a categoria de crescimento dos suínos.

1.2.10. Objetivos

Gerais – Avaliar o efeito do aumento das relações de aminoácidos (Met, Thr e Trp) para suínos imunologicamente desafiados e alimentados com o sistema de alimentação de precisão; e avaliar o efeito do aumento das relações Thr:Lys para suínos com diferentes taxas de crescimento e alimentados com o sistema de alimentação de precisão.

Específicos

- Avaliar a utilização da alimentação de precisão para suinos submetidos ao desafio imune.

- Avaliar o desempenho, composição corporal e balanço de N de suínos desafiados e alimentados com o sistema de alimentação de precisão e suplementados com aminoácidos funcionais (Met, Thr e Trp).

- Avaliar o efeito da suplementação de aminoácidos funcionais nos parâmetros sanguíneos de suínos sob desafio imunológico.

- Estudar o efeito do fornecimento de dietas com diferentes relações Thr:Lys no desempenho, composição corporal e balanço de nutrientes de suínos classificados em rápida, média e baixa taxa de crescimento na alimentação de precisão.

- Avaliar se a relação ótima de Thr:Lys na alimentação de precisão para suínos é afetada pela categoria de crescimento e se essa relação é diferente daquelas observadas em suínos alimentados com o sistema convencional por fases.

1.3. Referências

Andretta, I., Pomar, C., Rivest, J., Pomar, J., Lovatto, P. A., & Radünz Neto, J. (2014). The impact of feeding growing–finishing pigs with daily tailored diets using precision feeding techniques on animal performance, nutrient utilization, and body and carcass composition. **Journal of Animal Science**, 92(9), 3925-3936.

Andretta, I., Pomar, C., Rivest, J., Pomar, J., & Radünz, J. (2016). Precision feeding can significantly reduce lysine intake and nitrogen excretion without compromising the performance of growing pigs. **Animal**, 10(7), 1137-1147.

Aymerich, P., Soldevila, C., Bonet, J., Gasa, J., Coma, J., & Solà-Oriol, D. (2020). Increasing dietary lysine impacts differently growth performance of growing pigs sorted by body weight. **Animals**, 10(6), 1032.

Barcellos, J., Alves, W. J., Arnaut, P. R., Fonseca, L., Muniz, J. C. L., Dorigam, J. C. D. P., ... & Hannas, M. I. (2021). Assessment of digestible lysine requirements in lipopolysaccharide-challenged pigs. **Journal of Animal Science**, 99(12), skab336.

Beaulieu, A. D., Aalhus, J. L., Williams, N. H., & Patience, J. F. (2010). Impact of piglet birth weight, birth order, and litter size on subsequent growth performance, carcass quality, muscle composition, and eating quality of pork. **Journal of Animal Science**, 88(8), 2767-2778.

Cloutier, L., Pomar, C., Montminy, M. L., Bernier, J. F., & Pomar, J. (2015). Evaluation of a method estimating real-time individual lysine requirements in two lines of growing–finishing pigs. **Animal**, 9(4), 561-568.

Cooney, R. N., Kimball, S. R., & Vary, T. C. (1997). Regulation of skeletal muscle protein turnover during sepsis: mechanisms and mediators. **Shock**, 7(1), 1-16.

De Lange, C. F. M., Morel, P. C. H., & Birkett, S. H. (2003). Modeling chemical and physical body composition of the growing pig. **Journal of Animal Science**, 81(14_suppl_2), E159-E165.

De Ridder, K., Levesque, C. L., Htoo, J. K., & De Lange, C. F. M. (2012). Immune system stimulation reduces the efficiency of tryptophan utilization for body protein deposition in growing pigs. **Journal of Animal Science**, 90(10), 3485-3491.

Delmastro-Greenwood, M. M., & Piganelli, J. D. (2013). Changing the energy of an immune response. **American Journal of Clinical and Experimental Immunology**, 2(1), 30.

Dourmad, J. Y., Seve, B., Latimier, P., Boisen, S., Fernandez, J., Van der Peet-Schwering, C., & Jongbloed, A. W. (1999). Nitrogen consumption, utilisation and losses in pig production in France, The Netherlands and Denmark. **Livestock Production Science**, 58(3), 261-264.

Flachowsky, G., & Kamphues, J. (2012). Carbon footprints for food of animal origin: what are the most preferable criteria to measure animal yields?. **Animals**, 2(2), 108-126.

Garcia-Launay, F., Van der Werf, H. M. G., Nguyen, T. T. H., Le Tutour, L., & Dourmad, J. Y. (2014). Evaluation of the environmental implications of the incorporation of feed-use amino acids in pig production using Life Cycle Assessment. **Livestock Science**, 161, 158-175.

Grant, R. W., & Stephens, J. M. (2015). Fat in flames: influence of cytokines and pattern recognition receptors on adipocyte lipolysis. **American Journal of Physiology-Endocrinology and Metabolism**, 309 (3), E205-E213.

Hauschild, L., Pomar, C., & Lovatto, P. A. (2010). Systematic comparison of the empirical and factorial methods used to estimate the nutrient requirements of growing pigs. **Animal**, 4(5), 714-723.

Hauschild, L., Lovatto, P. A., Pomar, J., & Pomar, C. (2012). Development of sustainable precision farming systems for swine: estimating real-time individual amino acid requirements in growing-finishing pigs. **Journal of Animal Science**, 90(7), 2255-2263.

Huntley, N. F., Nyachoti, C. M., & Patience, J. F. (2018). Lipopolysaccharide immune stimulation but not β-mannanase supplementation affects maintenance energy requirements in young weaned pigs. **Journal of Animal Science and Biotechnology**, 9, 1-16.

Hotamisligil, G. S., Shargill, N. S., & Spiegelman, B. M. (1993). Adipose expression of tumor necrosis factor-α: direct role in obesity-linked insulin resistance. **Science**, 259 (5091), 87-91.

Johnson, R. W. (1997). Inhibition of growth by pro-inflammatory cytokines: an integrated view. **Journal of Animal Science**, 75(5), 1244-1255.

Johnson, R. W. (1998). Immune and endocrine regulation of food intake in sick animals. **Domestic animal endocrinology**, 15(5), 309-319.

Jones, C. K., & Patience, J. F. (2014). Variation in nutrient digestibility and energy intake are key contributors to differences in postweaning growth performance. **Journal of Animal Science**, 92(5), 2105-2115.

Kyriazakis, I., & Sandberg, F. B. (2006). The problem of predicting the partitioning of scarce resources during sickness and health in pigs. **In Mechanistic modelling in pig & poultry production** (pp. 117-142). Wallingford UK: CABI.

Le Floc'h, N., Melchior, D., & Obled, C. (2004). Modifications of protein and amino acid metabolism during inflammation and immune system activation. **Livestock Production Science**, 87(1), 37-45.

Le Floc'h, N., Melchior, D., & Sève, B. (2008). Dietary tryptophan helps to preserve tryptophan homeostasis in pigs suffering from lung inflammation. **Journal of Animal Science**, 86(12), 3473-3479.

Le Floc'h, N., Otten, W., & Merlot, E. (2011). Tryptophan metabolism, from nutrition to potential therapeutic applications. **Amino Acids**, 41, 1195-1205.

Le Floc'h, N., Wessels, A., Corrent, E., Wu, G., & Bosi, P. (2018). The relevance of functional amino acids to support the health of growing pigs. **Animal Feed Science and Technology**, 245, 104-116.

Lien, K. A., Sauer, W. C., & Fenton, M. (1997). Mucin output in ileal digesta of pigs fed a protein-free diet. **Zeitschrift für Ernährungswissenschaft**, 36(2), 182-190.

Li, P., Yin, Y. L., Li, D., Kim, S. W., & Wu, G. (2007). Amino acids and immune function. **British Journal of Nutrition**, 98(2), 237-252.

Li, H., Wan, H., Mercier, Y., Zhang, X., Wu, C., Wu, X., ... & Fang, Z. (2014). Changes in plasma amino acid profiles, growth performance and intestinal antioxidant capacity of piglets following increased consumption of methionine as its hydroxy analogue. **British Journal of Nutrition**, 112(6), 855-867.

Mahan, D. C., & Shields Jr, R. G. (1998). Essential and nonessential amino acid composition of pigs from birth to 145 kilograms of body weight, and comparison to other studies. **Journal of Animal Science**, 76(2), 513-521.

Mittal, M., Siddiqui, M. R., Tran, K., Reddy, S. P., & Malik, A. B. (2014). Reactive oxygen species in inflammation and tissue injury. **Antioxidants & redox signaling**, 20(7), 1126-1167.

Möhn, S., Gillis, A. M., Moughan, P. J., & De Lange, C. F. M. (2000). Influence of dietary lysine and energy intakes on body protein deposition and lysine utilization in the growing pig. **Journal of Animal Science**, 78(6), 1510-1519.

National Research Council. (2012). Nutrient requirements of swine.

Nissen, P. M., & Oksbjerg, N. (2011). Birth weight and postnatal dietary protein level affect performance, muscle metabolism and meat quality in pigs. **Animal**, 5(9), 1382-1389.

Ortiz, M. T., Arnaut, P. R., Valini, G. A. C., França, I., Silva, C. A., de Oliveira, M. J. K., ... & Hauschild, L. (2024). A salmonella-challenge impacts the variability of performance, body composition and lysine requirements of growing pigs under poor housing conditions. *Livestock Science*, 105462.

Paredes, S. P., Jansman, A. J. M., Verstegen, M. W. A., Awati, A., Buist, W., Den Hartog, L. A., ... & Gerrits, W. J. J. (2012). Analysis of factors to predict piglet body weight at the end of the nursery phase. **Journal of Animal Science**, 90(9), 3243-3251.

Pastorelli, H., Van Milgen, J., Lovatto, P., & Montagne, L. (2012). Meta-analysis of feed intake and growth responses of growing pigs after a sanitary challenge. **Animal**, 6(6), 952-961.

Pomar, C., Kyriazakis, I., Emmans, G. C., & Knap, P. W. (2003). Modeling stochasticity: dealing with populations rather than individual pigs. **Journal of Animal Science**, 81(14_suppl_2), E178-E186.

Pomar, C., Hauschild, L., Zhang, G. H., Pomar, J., & Lovatto, P. A. (2009). Applying precision feeding techniques in growing-finishing pig operations. **Revista Brasileira de Zootecnia**, 38, 226-237.

Pomar, C., Hauschild, L., Zhang, G. H., Pomar, J., & Lovatto, P. A. (2010). Precision feeding can significantly reduce feeding cost and nutrient excretion in growing animals. **In Modelling nutrient digestion and utilization in farm animals** (pp. 325-334). Wageningen Academic.

Pomar, C., Hauschild, L., Zhang, G. H., Pomar, J., & Lovatto, P. A. (2010). Precision feeding can significantly reduce feeding cost and nutrient excretion

in growing animals. **In Modelling nutrient digestion and utilization in farm animals** (pp. 325-334). Wageningen Academic.

Pomar, C., Pomar, J., Dubeau, F., Joannopoulos, E., & Dussault, J. P. (2014). The impact of daily multiphase feeding on animal performance, body composition, nitrogen and phosphorus excretions, and feed costs in growing–finishing pigs. **Animal**, 8(5), 704-713.

Pomar, C., & Remus, A. (2019). Precision pig feeding: a breakthrough toward sustainability. Animal Frontiers, 9(2), 52-59.

Popov, A., & Schultze, J. L. (2008). IDO-expressing regulatory dendritic cells in cancer and chronic infection. **Journal of Molecular Medicine**, 86(2), 145-160.

Reeds, P. J., Fjeld, C. R., & Jahoor, F. (1994). Do the differences between the amino acid compositions of acute-phase and muscle proteins have a bearing on nitrogen loss in traumatic states?. **The Journal of Nutrition**, 124(6), 906-910.

Rémond, D., Buffiere, C., Godin, J. P., Mirand, P. P., Obled, C., Papet, I., ... & Faure, M. (2009). Intestinal inflammation increases gastrointestinal threonine uptake and mucin synthesis in enterally fed minipigs. **The Journal of Nutrition**, 139(4), 720-726.

Remus, A., Hauschild, L., Corrent, E., Létourneau-Montminy, M. P., & Pomar, C. (2019a). Pigs receiving daily tailored diets using precision-feeding techniques have different threonine requirements than pigs fed in conventional phase-feeding systems. **Journal of Animal Science and Biotechnology**, 10, 1-17.

Remus, A., Pomar, C., Perondi, D., Gobi, J. P., da Silva, W. C., de Souza, L. J., & Hauschild, L. (2019b). Response to dietary methionine supply of growing pigs fed daily tailored diets or fed according to a conventional phase feeding system. **Livestock Science**, *222*, 7-13.

Remus, A., Hauschild, L., & Pomar, C. (2020). Simulated amino acid requirements of growing pigs differ between current factorial methods. **Animal**, 14(4), 725-730.

Rodrigues, L. A., Wellington, M. O., González-Vega, J. C., Htoo, J. K., Van Kessel, A. G., & Columbus, D. A. (2021). Functional amino acid supplementation,

regardless of dietary protein content, improves growth performance and immune status of weaned pigs challenged with Salmonella Typhimurium. **Journal of Animal Science**, 99(2), skaa365.

Sandberg, F. B., Emmans, G. C., & Kyriazakis, I. (2006). A model for predicting feed intake of growing animals during exposure to pathogens. **Journal of Animal Science**, 84(6), 1552-1566.

Sandberg, F. B., Emmans, G. C., & Kyriazakis, I. (2007). The effects of pathogen challenges on the performance of naïve and immune animals: the problem of prediction. **Animal**, 1(1), 67-86.

Santos, L. S. D., Pomar, C., Campos, P. H. R. F., da Silva, W. C., Gobi, J. D. P., Veira, A. M., ... & Hauschild, L. (2018). Precision feeding strategy for growing pigs under heat stress conditions. **Journal of Animal Science**, 96(11), 4789-4801.

Schaart, M. W., Schierbeek, H., van der Schoor, S. R., Stoll, B., Burrin, D. G., Reeds, P. J., & van Goudoever, J. B. (2005). Threonine utilization is high in the intestine of piglets. **The Journal of Nutrition**, 135(4), 765-770.

Smith, E. L., & Greene, R. D. (1947). Further studies on the amino acid composition of immune proteins. **Journal of Biological Chemistry**, 171, 355-362

Tokach, M. D., Goodband, R. D., DeRouchey, J. M., Dritz, S. S., & Nelssen, J. L. (2007). New tools to manage variability throughout the pork production chain. **In Proceedings of the London Swine Conference** (pp. 19-33).

Valini, G. A. C., Arnaut, P. R., França, I., Ortiz, M. T., Oliveira, M. J. K., Melo, A. D. B., ... & Hauschild, L. (2023). Increased dietary Trp, Thr, and Met supplementation improves growth performance and protein deposition of salmonella-challenged growing pigs under poor housing conditions. **Journal of Animal Science**, 101, skad141.

Van der Meer, Y., Lammers, A., Jansman, A. J. M., Rijnen, M. M. J. A., Hendriks, W. H., & Gerrits, W. J. J. (2016). Performance of pigs kept under different sanitary conditions affected by protein intake and amino acid supplementation. **Journal of Animal Science**, 94(11), 4704-4719.

Wu, G., Bazer, F. W., Wallace, J. M., & Spencer, T. E. (2006). Board-invited review: intrauterine growth retardation: implications for the animal sciences. **Journal of Animal Science**, 84(9), 2316-2337.

Zhang, G. H., Pomar, C., Pomar, J., & Del Castillo, J. R. (2012). L'alimentation de précision chez le porc charcutier: Estimation des niveaux dynamiques de lysine digestible nécessaires à la maximisation du gain de poids. **Journées de la Recherche Porcine**, 44, 171-176.

CAPÍTULO 2 – The effect of precision feeding and functional amino acids supplementation in *Salmonella*-challenged growing pigs under poor housing conditions

Este capítulo foi redigido de acordo com as normas da revista **Journal of Animal Science**

Running head: precision feeding and AA for challenged pigs

The effect of precision feeding and functional amino acids supplementation in *Salmonella*-challenged growing pigs under poor housing conditions.

Pedro Righetti Arnaut[†], Graziela Alves da Cunha Valini[†], Ismael França[†], Manoela Trevisan Ortiz[†], Marllon José Karpeggiane de Oliveira[†], Danilo Alves Marçal[†], Antonio Diego Brandão Melo[†], Amanda Faria de Oliveira[†], Henrique Gastmann Brand[‡], John Kyaw Htoo[§], Candido Pomar[¶], Aline Remus[¶], Luciano Hauschild[†*].

*Corresponding author: luciano.hauschild@unesp.br

[†]São Paulo State University (UNESP), School of Agricultural and Veterinary Sciences, Jaboticabal, São Paulo, Brazil, 14884-900.

[‡]Evonik Brasil Ltda, São Paulo, Brazil, 04711-904.

[§]Evonik Operations GmbH, Hanau, Germany, 63457.

[¶]Sherbrooke Research and Development Centre, Agriculture and Agri-Food Canada, 2000 Rue College, Sherbrooke, Quebec, Canada, J1M 0C8.

[1]The authors acknowledge the financial support received from the São Paulo Research Foundation (FAPESP, grant numbers 2018/15559-7 and 2019/10843-1), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES – Finance Code 001), Brazilian National Council for Scientific and Technological Development (CNPq, grants number 408287/2021-7, 308779/2023-1, and 442675/2023-2), Evonik Operations GmbH, and Seara Alimentos - JBS Foods Brazil.

Lay Summary

The immune system activation modifies the energy and amino acid metabolism of pigs and frequently reduces growth performance and nutrient utilization efficiency. The supplementation of individual precision fed challenged pigs with functional amino acids (threonine, methionine and tryptophan) was shown to increase nutrient efficiency and growth performance. The aim of this study was to evaluate the effect of precision feeding and functional amino acids supplementation on growth performance, body composition, nutrient efficiency, and blood parameters of pigs raised in poor sanitary conditions. The results indicate that the association between the current precision feeding model and functional amino acids supplementation do not increase nutrient efficiency nor reduces the negative impacts of the immune system activation. The benefits of supplementing precision fed pigs with functional amino acids may depend on ratios of functional amino acids used, the sanitary challenge intensity, and functional amino acid intake. Modifications in the precision feeding model should be performed to allow its utilization for immune challenged pigs. This study highlights the need to take into account the utilization of nutrients by the immune system when determining the nutritional requirements of animals.

Teaser Text

Precision feeding and functional amino acids supplementation did not increase nutrient efficiency nor mitigate the negative effects of the immune system activation in growing pigs. The new knowledge acquired in this study allows the improvement of our ability to

precisely feed immune challenged pigs and highlights the need of considering the nutrient utilization by the immune system in the pigs' requirements.

Abstract: Immune system activation impair pig' growth and increase population variability. Individual precision feeding (**IPF**) takes into account the variability between pigs and improve the efficiency of nutrient utilization. Additionally, functional amino acids (**FAA**) supplementation reduces the negative impacts of the immune system activation. However, the combined effect of IPF and FAA supplementation for immune challenged pigs remains to be evaluated. We hypothesized that the combined effect of IPF and FAA supplementation would increase nutrient efficiency and reduce the effects of the immune activation on pig performance. Therefore, the objective of this study was to evaluate the use of IPF and FAA supplementation on growth performance, body composition, nutrient efficiency and blood parameters of growing pigs raised in different sanitary conditions (**SC**). A total of 120 female pigs were used in two experiments. The pigs were allotted in a good (**GSC**) or poor (**PSC**) sanitary condition barn (60 pigs each). The GSC was established with good biosecurity protocols. In the PSC, pigs were orally inoculated with *Salmonella* Typhimurium and manure from a commercial swine farm was spread on the pen floor. In each SC barn, pigs were randomly assigned to a 2 × 2 factorial arrangement. The treatments consisted of two feeding systems (**FS**) (group-phase feeding [**GPF**] and IPF) and two diets (control with 100% [**CN**] or supplemented with 120% of the SID Met + Cys, Thr and Trp to Lys ratios [**AA+**] above InraPorc recommendations). Pigs had *ad libitum* access to water and feed during the 28-d experimental period. The PSC increased rectal temperature and haptoglobin concentration compared to d 0 ($P < 0.05$). The IPF

impaired the growth performance and body composition in GSC and PSC ($P < 0.05$). The performance of IPF pigs increased when fed AA+ diets in the GSC, but no dietary effects were observed in the PSC ($P > 0.05$). Group-phase feeding pigs ingested more SID Lys in both SC but had the same Lys efficiency of IPF in the PSC ($P > 0.05$). Large fluctuations in body weight and daily feed intake might have impaired the IPF model's ability to estimate the SID Lys requirements. The absence of the FAA effect in PCS pigs might be related to the FAA ratios used in the diets, the immune challenge intensity, and FAA intake. In conclusion, FAA supplementation in IPF fed pigs did not alleviate the negative impact of the immune challenge in growth performance nor increase nutrient efficiency.

Keywords: amino acids, body composition, immune challenge, individual feeding, nutrient efficiency

Abbreviations

AA, amino acids

CFU, colony forming units

D, diet

DFI, daily feed intake

DG, daily gain

FAA, functional amino acids

FS, feeding system

LD, lipid deposition

Nal+, nalidixic acid

NE, net energy

PBS, phosphate buffer saline

PD, protein deposition

SC, sanitary condition

STTD, standard total tract digestible

1. Introduction

In swine farms, pigs are constantly exposed to pathogens and unsanitary conditions that activate their immune system. The release of pro-inflammatory cytokines changes the metabolism and redistribute nutrients away from growth to sustain the immune response (Johson et al., 1997; Huntley et al., 2018). Consequently, protein and lipid deposition diminishes to increase the amount of nutrients available for the immune system which, in turn, limit growth performance and nutrient efficiency (Huntley et al., 2018; Valini et al., 2023). The extent of the immune activation impact on growth depends on the pathogen dosage, virulence and the individual pig coping abilities (Wellock et al., 2004; Sandberg et al., 2006; 2007). Therefore, the variability of the nutrient requirement (Ortiz et al., 2024) as well as the demand of some nutrients (Valini et al., 2023) increase during the immune system activation.

Conventionally, pigs are fed with group-phase feeding (**GPF**) programs that provide a single diet for a group of pigs during a phase of the production cycle. These programs estimate the dietary concentration based on the requirement of the average pig (Hauschild et al., 2010). However, nutritional requirements vary between pigs and over-time (Pomar et al, 2011; Andretta et al., 2016). These variations are not addressed by GPF,

resulting in most pigs being overfed and reducing their nutrient efficiency (Hauschild et al., 2010; Pomar et al, 2011; Andretta et al., 2016). Once the nutrient requirement variability increases, this limitation further impacts in the nutrient utilization of immune challenged pigs.

Emerging tools, such as the individual precision feeding (**IPF**) address this variation by providing tailored diets that match the daily nutritional requirement of each individual animal (Hauschild et al., 2012). Studies have shown that feeding pigs individually with IPF significantly reduced the Lys intake by 27% and nitrogen excretion by 30% without compromising growth (Andretta et al., 2014; 2016). These findings suggest that IPF could be a promising feeding strategy to enhance nutrient efficiency in immune-challenged pigs. However, the use of IPF for immune-challenged pigs remains to be evaluated.

Additionally, the metabolic changes resulted from the immune activation increase the need of specific amino acids (Reeds et al., 1994; Le Floc'h et al., 2018). These amino acids are often referred to as "functional" AA (**FAA**) due to their role in the synthesis of immune components. For instance, the supplementation of the FAA Met, Thr and Trp was shown to mitigate the impact of the immune stimulation (van der Meer et al., 2016; Rodrigues et al., 2018; Valini et al., 2023). These FAA, improve the antioxidant status, prevent pathogen translocation and minimize the long-lasting effects of the immune response, respectively (Le Floc'h et al., 2018; Rodrigues et al., 2021). Therefore, the association of IPF and FAA could increase the performance of immune challenged pigs and increase their nutrient efficiency.

We hypothesized that the combined effect of IPF and FAA would reduce the effects of the immune system activation on pig performance and increase nutrient efficiency. Thus, the objective of this study was to evaluate growth performance, body composition, Lys and N efficiency and blood parameters of sanitary-challenged pigs fed with different feeding systems (**FS**), with or without FAA supplementation.

2. Material and Methods

All experimental procedures applied in this trial followed the Brazilian National Council of the Control of Animal Experimentation (CONCEA) and were reviewed and approved [protocol nº 0607/22] by the Ethical Committee on Animal Use (CEUA) of the School of Agricultural and Veterinary Sciences of the São Paulo State University (UNESP).

2.1 Animal husbandry and experimental design

Two studies with two different sanitary conditions (**SC**) were conducted simultaneously at the Swine Research Facility of São Paulo State University, Brazil. One hundred and twenty female pigs (Pietran × [Large White × Landrace]) with initial BW of 16.1 ± 2.1 kg were divided and housed in two similar growing-finishing barns with 60 pigs each. Each facility consisted of a single pen with concrete floors and controlled temperature equipped with eight nipple drinkers and five automated feeding stations (Automatic Intelligent Precision Feeders; University of Lleida, Spain; Pomar et al., 2011). The feeders can identify the pigs through electronic ear tags with single identification numbers, allowing the pigs to be housed in the same pen and receive the prescribed dietary treatment.

Additionally, the feeders can mix up to four different diets according to proportions that are registered in its software and register the individual feed intake. The feeders were calibrated and tested every day during the adaptation and experimental periods. A 14 d period was used to adapt the pigs to the feeders and collect individual baseline information on daily feed intake (**DFI**), BW and daily gain (**DG**). After, the adaptation period, the barns were used to create either a poor (**PSC**) or good (**GSC**) sanitary condition. Within the GSC and PSC, the pigs (23.15 ± 2.81 and 22.82 ± 2.78 kg BW, respectively) were randomly distributed into blocks of BW in a 2 × 2 nested factorial arrangement. The treatments consisted of two feeding systems: GPF and IPF; and two diets: control (**CN**) or supplemented (**AA+**) with Met + Cys, Thr and Trp at 120% of InraPorc (van Milgen et al., 2008) recommendations.

Before the beginning of the trials, rectal swabs were individually performed in all pigs to screen for the presence of *Salmonella* species. Therefore, only negative pigs were used in the trials. A 12 h period of light was set from 0700 h to 1900 h and the room temperature was gradually decreased from 22 °C at the beginning to 18 °C at the end of the experimental period which lasted 28 days. During the adaptation and experimental periods, the pigs had *ad libitum* access to water and feed.

2.2 Sanitary conditions

The barns were used to create two different SC. All pigs in the PSC were induced to an infection on d 0 through oral inoculation of 5 mL solution of brain heart infusion broth (CM 1135, Oxoid, Thermo Fisher Scientific, England) containing a total of 2×10^9 CFU of *Salmonella* Typhimurium. Additionally, fresh manure from a commercial pig farm

was evenly spread on the pen floor (1.7 kg/m^2; Valini et al., 2023). The strain *Salmonella enterica* subsp. enterica serovar Typhimurium (RL0971/09) was originally isolated from swine feces and it was naturally resistant to nalidixic acid (**Nal+**; 25 µg/mL). The inoculum was prepared according to Wood et al. (1991) and Oliveira et al. (2010) at the Laboratory of Ornitopathology (Department of Veterinary Pathology, FCAV/UNESP, Jaboticabal-SP, Brazil) 72 h before inoculation at 37°C in buffered peptone water and diluted with sterile PBS. The solution was inoculated by oral gavage after six hours of fasting and no water consumption for one hour. The PSC barn was not cleaned during the experimental period. On the other hand, the GSC barn was cleaned twice a day with pressurized water. Disinfected clothing and footwear were required before entering the barn. In the GSC, pigs received an oral inoculation of 5 mL of brain heart infusion broth without *Salmonella* Typhimurium on d 0 to mimic the same handling procedure applied to PSC pigs. Rectal swabs were performed weekly in all GSC pigs to screen for the presence of the *Salmonella* Typhimurium strain inoculated in the PSC condition to monitor a possible cross-contamination. Pigs in both conditions did not receive any medication or preventive treatments before or during the trial.

2.3 Feeding systems and diets

The pigs were randomly assigned to GPF or IPF feeding systems on d 0 in both SC. Group-phase pigs were fed with a diet containing 1.11% SID Lys during the whole trial. The SID Lys concentration of GPF pigs corresponded to the requirement of the animal placed in the 80th percentile. The 80th percentile pig was chosen assuming that its SID Lys requirement represents the dietary concentration that maximizes the population growth

performance (Hauschild et al., 2010; Remus et al., 2020). To determine the 80th percentile for this trial, we used the average of the 80th percentile of all pigs during the three days before the beginning of the trial. Pigs from both GSC and PSC allotted in the GPF treatment received the same SID Lys concentration. On the other hand, pigs allotted in the IPF group received tailored diets adjusted to its daily SID Lys requirement according to the model of Hauschild et al. (2012). The model uses an empirical and a mechanistic component to estimate the SID Lys requirements of each individual. The empirical component forecast the DFI, BW and DG based on the feed intake and growth patterns of each pig. The mechanist component uses the forecasted DFI, BW and DG as input in factorial equations to estimate the SID Lys requirement for maintenance and growth (Hauschild et al., 2012). Lysine requirements for maintenance were estimated adding basal endogenous losses (0.313 g Lys/kg DM × DFI) to the losses related to desquamation in the digestive tract (0.0045 g Lys/kg $BW^{0.75}$ × $BW^{0.75}$), and losses related to body protein turn over (0.0239 g Lys/kg $BW^{0.75}$ × $BW^{0.75}$; van Milgen et al., 2008). The SID Lys requirements for growth were estimated assuming that 16% of the DG is protein (de Lange et al., 2003) and that 7% of the protein deposition is composed of Lys (Mahan and Shields, 1998), and that the efficiency of Lys retention from dietary digestible Lys is 72% (Möhn et al., 2000). Body weight was recorded twice a week and DFI measured daily by the feeding stations to be used as input in the IPF model.

Four experimental diets were formulated for this trial: A1, B1, A2 and B2 (Table 1). Diets A (high nutrient density) were formulated to meet the requirement of the most demanding pig at the beginning of the trial (1.30% SID Lys). Meanwhile, diets B (low nutrient density) were formulated to meet the requirements of the least demanding pig at

the end of the trial (0.47% SID Lys). Diets A1 and B1 were formulated with 100% (**CN**) and diets A2 and B2 with 120% (**AA+**) of the SID Met + Cys, Thr and Trp:Lys. Therefore, the SID Lys requirements of the pigs in the GPF and IPF treatments were achieved with either a blend of CN (A1 and B1) or AA+ (A2 and B2). Amino acids were balanced in the CN diets in fixed proportions relative to SID Lys, with ratios of 30% Methionine, 60% Methionine + Cysteine, 65% Threonine, 20% Tryptophan, 60% Isoleucine, 100% Leucine, 70% Valine, 50% Phenylalanine, 32% Histidine, and 42% Arginine (van Milgen et al., 2008). Minerals (Ca, P and Na) and energy content were calculated using the NRC (2012) recommendations for the phase from 25 to 50 kg BW. The diets were steam-pelleted (2.5 mm) and no in-feed antibiotics were added.

Dietary total AA and CP concentrations were analyzed using the Association of Official Analytic Chemists (AOAC, method 994.12, 2010) at the AMINOLab of Evonik (Germany). The estimated SID AA composition was estimated applying the digestibility coefficients obtained with the EvaPig® on the analysed total AA concentration (Table 1).

Table 1. Ingredients and chemical composition of the experimental diets A1, A2, B1 and B2 (as-fed basis)[1].

Item, %	A1	A2	B1	B2
Feedstuff				
Corn	67.7	67.1	90.8	92.1
Soybean meal	26.0	26.1	-	-
Soybean oil	1.27	1.33	0.54	0.05
Kaolin (inert)	-	-	4.85	3.97
Dicalcium phosphate	1.41	1.41	1.22	1.21
Limestone	0.77	0.77	0.72	0.73
Salt	0.22	0.22	0.23	0.23
L-lysine[2]	0.86	0.86	0.49	0.49
DL-methionine[2]	0.29	0.45	0.01	0.06

L-threonine[2]	0.29	0.46	0.09	0.15
L-tryptophan[2]	0.09	0.14	0.05	0.07
L-valine	0.18	0.18	0.03	0.02
L-isoleucine	0.12	0.12	0.05	0.05
Maltodextrin	0.50	0.50	0.50	0.50
Min/vit premix[3]	0.12	0.12	0.12	0.12
Antifungic	0.10	0.10	0.10	0.10
Choline chloride	0.06	0.06	0.06	0.06
Antioxidant	0.01	0.01	0.01	0.01
Mycotoxin adsorbent	0.10	0.10	0.10	0.10
Calculated chemical composition				
Dry matter	83.54	82.95	84.65	84.40
Crude protein	18.36	18.61	7.96	8.15
ME, kcal/kg	3,360	3,370	3,150	3,170
NE, kcal/kg	2,480	2,480	2,480	2,480
Total Ca	0.74	0.74	0.59	0.59
Total P	0.55	0.55	0.41	0.41
STTD P	0.34	0.34	0.27	0.27
Estimated SID AA[4]				
Lysine	1.31	1.28	0.50	0.51
Methionine + Cysteine	0.77	0.91	0.30	0.36
Threonine	0.84	1.02	0.34	0.41
Tryptophan[5]	0.26	0.31	0.09	0.11
Methionine	0.51	0.66	0.15	0.19
Cysteine	0.24	0.25	0.16	0.15
Valine	0.86	0.85	0.40	0.37
Arginine	0.94	0.95	0.38	0.35
Isoleucine	0.71	0.71	0.32	0.30
Leucine	1.29	1.32	0.90	0.85
Histidine	0.41	0.41	0.22	0.21
Phenylalanine	0.71	0.72	0.37	0.35

[1]Diets A and B were formulated with 1.30% and 0.47% SID Lys, respectively. Diets 1 and 2 were, respectively, formulated with 100% and 120% of the Met + Cys, Thr and Trp:Lys recommended by the InraPorc (van Milgen et al., 2008).

[2]Amino acids BioLys®, MetAMINO®, ThreAMINO®, TrypAMINO® were provided by Evonik Nutrition & Care GmbH (Hanau-Wolfgang, Germany). Supplied per kg of product (as-fed basis): 62.4%, 99.0%, 98.5% and 98.0% of L-lysine, DL-methionine, L-threonine and L-tryptophan, respectively.

[3]Mineral and vitamin premix provided by Nutri – Guabi Nutrição e Saúde Animal (Sales Oliveira-São Paulo, Brazil). Supplied per kg of diet (as-fed basis): Manganese, 40 mg; Copper, 15 mg; Iron, 24.93 mg; Cobalt, 0.168 mg; Iodine, 1.416 mg; Zinc, 74.971 mg; Folic acid, 0.32 mg; D-Pantothenic acid, 14.8 mg; Biotin, 0.04 mg; Niacin, 28 mg; Selenium, 0.25 mg; Vitamin A, 6000 IU; Vitamin B_1, 1.2 mg; Vitamin B_{12}, 22 mcg; Vitamin B_2, 4.4 mg; Vitamin B_6, 1.4 mg; Vitamin D_3, 1400 IU; Vitamin E, 26 IU; Vitamin K_3, 2.16 mg.

[4]Estimated applying the digestibility coefficients obtained with the EvaPig® on the total AA concentrations.

[5]Estimated values used to formulate the diets.

SID = standardized ileal digestible, STTD = standardized total tract digestible.

Values are presented on as-fed basis.

2.4 Data collection

Rectal temperature, blood sampling, body composition and nutrient efficiency analyses were carried out on ten pigs per treatment. Selection criteria involved choosing pigs with the closest body weights to the average. All procedures were conducted in both SC on the same experimental day.

2.4.1 Growth performance

The BW and DFI were measured twice a week and daily, respectively. These data were used as input to estimate the requirement of the 80th percentile and IPF requirements. For the growth performance analysis, the BW and feed intake were used to assess the ADG, ADFI and G:F.

2.4.2 Rectal temperature and fecal Salmonella shedding

Rectal temperature was measured daily using digital thermometers (Accumed-Glicomed, Rio de Janeiro, Brazil) from d 0 to d 7. On d 0, the temperature was measured before introducing the pigs to the SC and dietary treatments.

Fecal samples were collected from all PSC pigs on d 3, 7, 14, 21 and 28 for the detection of *Salmonella*. The samples were individually collected with rectal stimulation. Fresh fecal samples were serially diluted in phosphate buffer saline (**PBS**) (1:10) until they reached the final concentration of 10^{-6}. One hundred microliters from each dilution were plated on brilliant green agar containing Nal+. Plates were incubated at 37 °C for 24 h. In the absence of growth, an equal volume of Rappaport broth (CM0669, Oxoid, United Kingdom), prepared in double concentration, was added to the falcon tubes containing the homogenized sample in PBS (1:10). The sample tubes were incubated at 37 °C for 24 h and plated again in Nal+ at 37 °C for 24 h. The number of CFU per gram of feces was transformed into Log10 for further analysis. Plates with at least one colony of *Salmonella* Typhimurium were considered positive and were used to determine the percentage of positive pigs in each treatment. Meanwhile, the rectal swabs collected before and during the trial to screen for the presence of *Salmonella* Typhimurium were serially diluted in (1:10) until they reached the final concentration of 10^{-6}. From each dilution, 0.1 mL was plated on brilliant green agar (CM0263, Oxoid, England) and incubated at 37 °C for 24 h.

2.4.3 Blood sampling and analysis

Blood samples were collected from the jugular vein on d 0, 7 and 28, after a minimum of 6 hours fasting. On d 0, the samples were collected before introducing the pigs to the SC, feeding systems and diets. In each sampling, serum tubes (BD, New Jersey,

USA) were collected for haptoglobin, albumin, total protein, urea, creatinine, IgA, IgG and triglycerides analysis. The tubes were allowed to clot for 2 hours at room temperature before centrifugation for 10 min at 3000 × g at 4 °C and stored at −80 °C. The haptoglobin and albumin serum concentration were determined with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Weber and Osborn, 1969). The molecular weight and protein fraction concentrations were determined by computer densitometry (Shimadzu 9301, Kyoto, Japan) with a simple scanner. Biomarkers were used for protein identification (Sigma Marker, Sigma-Aldrich Biotechnology, Germany). The reference curves for densitometry evaluation of protein bands were made from the reading of the standard marker. After, the serum haptoglobin, albumin, IgA and IgG concentration were corrected using the serum total protein analysis. The serum concentration of total protein, urea, creatinine, and triglycerides were determined by the biuret method (Labtest®, Lagoa Santa, Brazil).

2.4.4 Body composition analysis

Body composition was estimated in the beginning and end of the experimental period. The pigs were anesthetized after 6 hours of fasting by intramuscular injection of xylazine (1.5 mg/kg BW) and ketamine (15 mg/kg BW) and scanned in prone position with dual-energy X-ray absorptiometry equipment ([**DXA**], GE Lunar Prodigy Advance; GE Healthcare, USA). The estimated lean and fat masses were converted into its protein and lipid chemical equivalents according to Pomar and Rivest (1996). This data was used to determine the body protein and lipid mass. Daily protein (**PD**) and lipid (**LD**) deposition

were calculated as the difference between the respective body constituents estimated by the DXA at the beginning and end of the experimental period.

2.4.5 Lysine and N utilization efficiencies

Lysine and N utilization efficiencies were obtained using only the pigs that were scanned with the DXA. Nitrogen and SID Lys intake were calculated multiplying the individual ADFI by its respective concentration in the blend of feeds provided. Nitrogen intake and retention were estimated assuming that dietary CP and PD contains 6.25% of N. Values of N efficiency were expressed as the percentage of the intake that was retained. Lysine efficiency was calculated by dividing the amount of available and retained Lys. Available Lys was estimated subtracting the maintenance from intake. Lysine maintenance requirements were calculated using the maintenance formulas to estimate the individual requirements (see *2.3 Feeding systems and diets*). Lys retention values were estimated assuming that 7% of the PD is Lys (Mahan and Shields, 1998).

2.5 Statistical analysis

The statistical analysis was conducted separately for each SC. Data were tested for normality using the UNIVARIATE procedure of SAS (version 9.4, SAS Institute Inc., North Carolina, USA) and analyzed using the MIXED procedure as a complete randomized block design. Feeding systems, diets and their interactions were included as fixed effects while the blocks of BW were included as a random effect in the statistical model. When significant, FS, diets and their interactions were compared with the F test. A Logistic regression and the Wilcoxon Rank Sum test were used to analyze the frequency and the

excretion of *Salmonella* Typhimurium, respectively. Blood parameters, rectal temperature, final BW, ADG, ADFI, SID Lys intake, final body protein and final body lipids were evaluated as repeated measures over time using the co-variance matrix with the lowest AIC value. Different periods of time were used in the repeated measures variable analysis. The sampling days were used for blood parameters and rectal temperature. The initial and final experimental days were used as fixed effects for the final BW, final body lipid and final body protein analysis. Finally, weekly values of ADG, ADFI and SID Lys intake were used as fixed effect in their respective analysis. The effect of Time was compared with the Tukey test. The individual pig was considered the experimental unit. Effects were considered significant when $P \leq 0.05$.

3. Results

3.1 General overview

Clinical visual examination showed that most pigs in the PSC had nausea, diarrhea and lethargy in the first week. During the experimental period, three pigs from the PSC were removed from the experiment due to lameness and arthritis. They were medicated following veterinarian guidelines and removed from the statistical analysis. All swabs performed in the GSC pigs were negative for the *Salmonella* Typhimurium strain inoculated in the PSC (data not shown), indicating the absence of cross-contamination between SC.

Descriptive analysis showed potential differences across SC in dietary SID Lys requirement patterns for IPF pigs (Fig. 1). The CV of IPF pigs' individual requirements during the trial were smaller in GSC (19%) than PSC (24%) pigs. The sanitary challenge

imposed to PSC pigs reduced their ADFI in both feeding systems when compared to the week before the trial (1027 g/d vs. 637 g/d). As a result, the average SID Lys concentration estimated for PSC pigs in the first week increased when compared to GSC (1.17% vs. 0.98% SID Lys, respectively). However, even with the increased SID Lys concentration, GSC pigs fed with GPF and IPF feeding methods ingested, respectively, 35% and 26% more SID Lys than PSC pigs within the same feeding system.

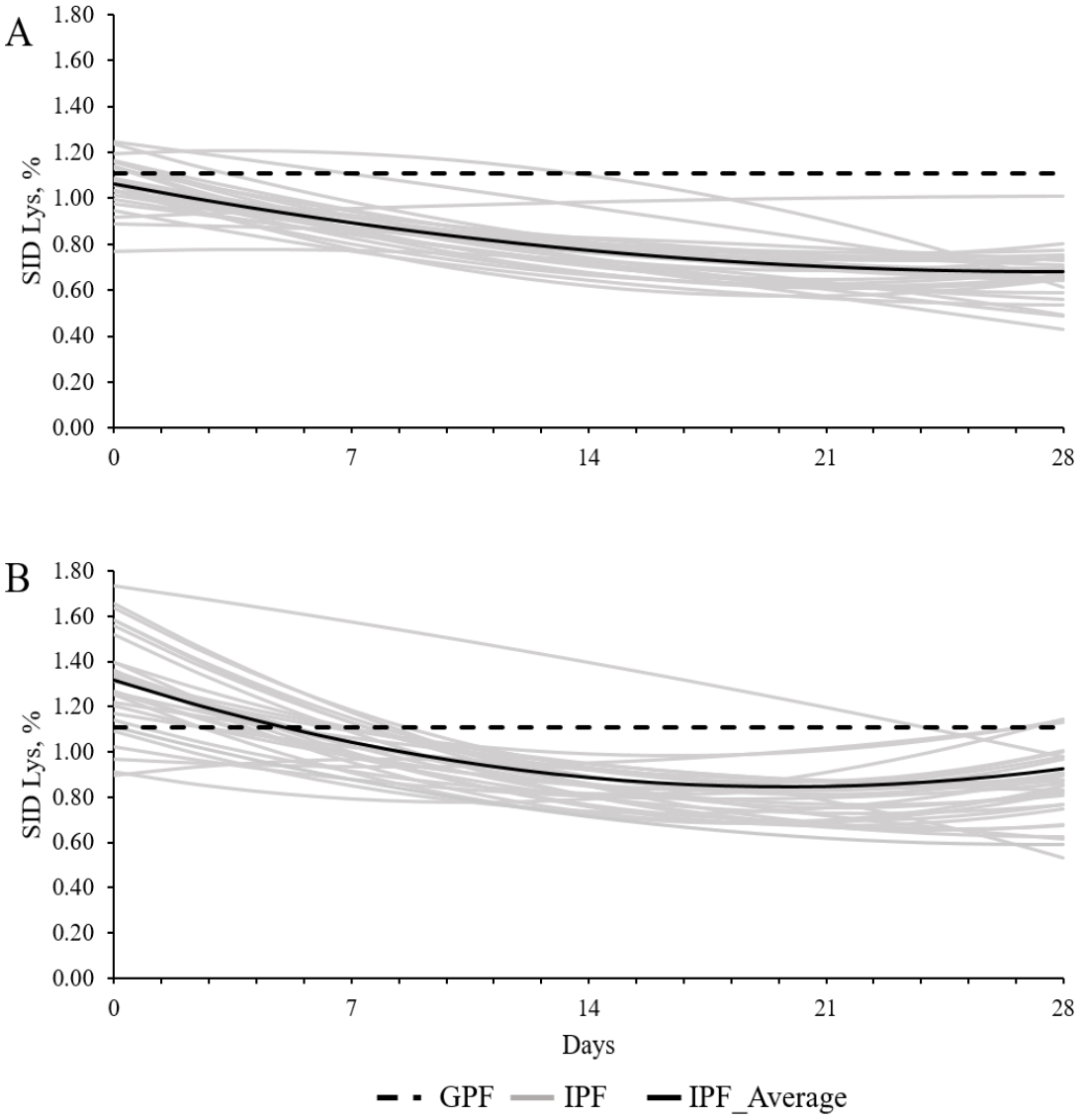

Figure 1. Estimated dietary SID Lys concentration requirements in group-phase (GPF) or individual precision feeding (IPF) systems for pigs raised in good (A) or poor (B) sanitary conditions. Pigs fed with GPF received a 1.11% SID Lys concentration diet overall the trial in both sanitary conditions. IPF pigs were individually fed with daily tailored diets to meet their individual requirements in both sanitary conditions. The good sanitary condition was achieved with biosecurity protocols whereas, in the poor condition, pigs were orally inoculated with *Salmonella* Typhimurium and manure from a commercial swine farm was spread on the pen floor.

Descriptive analysis showed that, during the 28-d trial, PSC pigs within GPF and fed with AA+ diets consumed 7% less SID Lys and 12%, 14% and 13% more SID Met + Cys, Thr and Trp than CN pigs, respectively. However, the ADFI of GPF pigs fed AA+ diets was 99 g lower than CN pigs during the first experimental week (709 g/d vs. 610 g/d, respectively). Consequently, in the first experimental week, AA+ pigs fed with GPF ingested 15% less SID Lys and only 2%, 5% and 3% more SID Met + Cys, Thr and Trp than CN pigs, respectively. On the other hand, in the overall 28-d trial, IPF pigs fed with AA+ diets within the PSC consumed 7%, 30%, 33% and 32% more SID Lys, Met + Cys, Thr and Trp when compared to CN pigs, respectively. Similar results were observed in the first experimental week with IPF pigs fed with AA+ diets ingesting 4%, 26%, 29% and 27% more SID Lys, Met + Cys, Thr and Trp than CN pigs, respectively.

3.2 Rectal temperature and Salmonella Typhimurium shedding

No differences ($P > 0.05$) in rectal temperature, percentage of *Salmonella* positive pigs, and *Salmonella* Typhimurium shedding (Fig. 3) were observed for the main effects of FS, diet and their interactions (Fig. 2). The GSC pigs' rectal temperature remained stable during the first week of the trial, declining on day 7 ($P < 0.05$). Conversely, PSC pigs'

rectal temperature sharply increased on d 1 after the inoculation with a graded reduction until d 7 ($P < 0.05$).

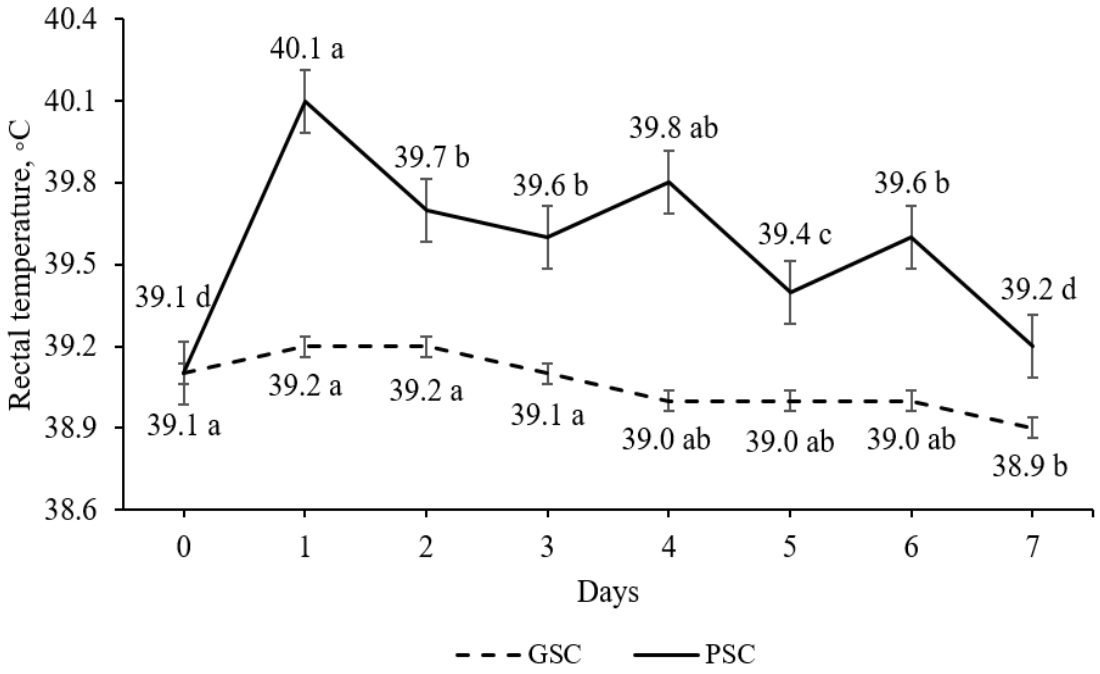

Figure 2. Rectal temperature of pigs allotted in good (GSC) or poor (PSC) sanitary conditions during the first week of trial. The good sanitary condition was achieved with biosecurity protocols whereas, in the poor condition, pigs were orally inoculated with *Salmonella* Typhimurium and manure from a commercial swine farm was spread on the pen floor. Different letters indicate differences between days in each sanitary condition ($P < 0.05$). No significant effects of feeding systems, diets and their interactions were observed ($P > 0.05$).

In the PSC, all pigs were at least one day positive for *Salmonella* Typhimurium shedding (Fig. 3). The highest percentages of positive pigs were observed on d 3 and 7 ($P < 0.05$) with a significant decrease on d 14, 21 and 28 ($P < 0.05$). Similarly, the *Salmonella* shedding decreased linearly towards the end of the experimental period ($P < 0.05$).

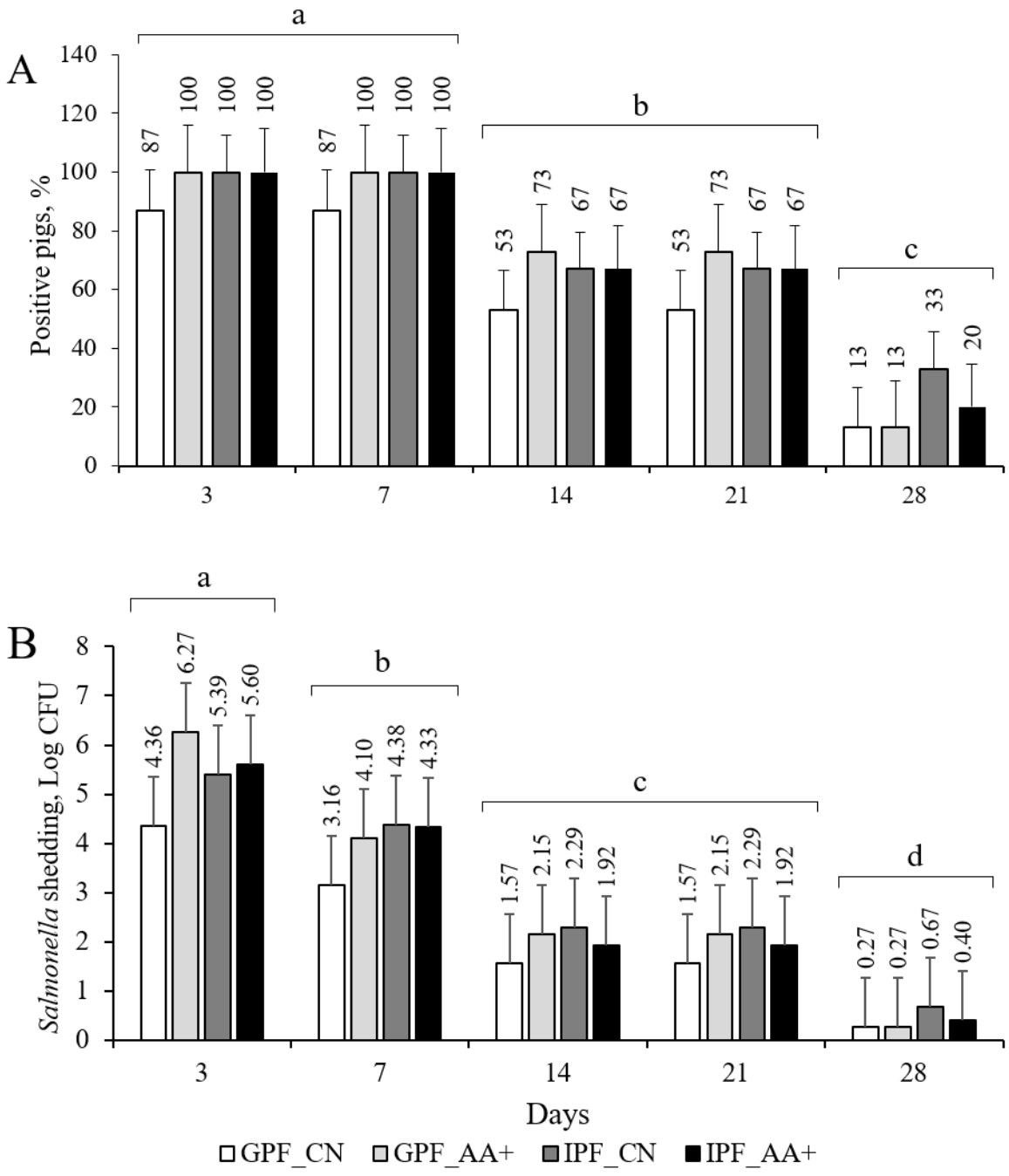

Figure 3. Percentage of positive pigs to *Salmonella* Typhimurium shedding (A) and Log of colony forming units (CFU) of *Salmonella* Typhimurium shedding (B) at five sampling days of poor sanitary condition pigs. The poor condition was achieved with oral inoculation of *Salmonella* Typhimurium and manure from a commercial swine farm was spread on the pen floor. Pigs were fed with either a control (CN) or functional AA supplemented (AA+) diet within group-phase (GPF) or individual precision feeding (IPF) systems. Different letters indicate differences between days ($P < 0.05$). No significant effects of feeding systems, diets and their interactions were observed ($P > 0.05$).

3.3 Animal performance and body composition

3.3.1 Good sanitary conditions

Growth performance and body composition of pigs raised in GSC or PSC are shown in Table 2. Interactions between feeding systems and time were observed for ADG and SID Lys intake in GSC and PSC ($P < 0.05$; Fig. 4). In the GSC, a triple interaction between feeding systems, diet and time demonstrated that, although IPF pigs had lower BW, it increased on d 28 when they were fed with AA+ diets ($P < 0.05$). Similarly, AA+ diets improved the ADG and G:F of IPF pigs ($P < 0.05$) in the same 28-d period. Body protein was greater in GPF pigs ($P < 0.05$) regardless of the dietary treatment. The ADFI was not affected ($P > 0.05$) by the FS or FAA supplementation. Main effects of feeding systems and dietary treatments were observed for PD where group-phase pigs had higher PD than IPF ($P < 0.05$) and AA+ pigs greater PD than pigs fed with CN diets ($P = 0.05$).

In the GSC group the SID Lys intake (g/d) of IPF pigs increased from the first to the second week ($P < 0.05$) and from the third to the fourth week ($P < 0.05$). Meanwhile, the SID Lys intake of GPF pigs placed in the GSC increased weekly ($P < 0.05$), reaching its highest amount at the last experimental week ($P < 0.05$). As a result, GPF pigs ingested more ($P < 0.05$) SID Lys than IPF pigs during the second (28%), third (52%) and fourth (62%) experimental week ($P < 0.05$). Group-phase and IPF pigs had similar ADG during the first and second week of trial ($P > 0.05$). However, during the third and fourth week, the ADG of GPF pigs were 35% and 27% higher than IPF pigs, respectively ($P < 0.05$). The G:F of GPF pigs remained stable during the entire experimental period ($P > 0.05$).

Conversely, the G:F of IPF pigs decreased during the third week ($P < 0.05$) and had a lower ($P < 0.05$) value compared to GPF pigs during the third week.

3.3.2 Poor sanitary conditions

No effects of diets or interactions between diets and feeding systems or diet and time were observed in the PSC ($P > 0.05$). Interactions ($P < 0.05$) between feeding systems and time were observed for BW, body lipid and body protein, demonstrating that the evolution of these variables over-time were different between pigs fed with GPF or IPF. Indeed, BW, body lipid and body protein were impaired when pigs were fed with IPF ($P < 0.05$). Main effects of the feeding system were observed for PD and LD in which IPF pigs had lower ($P < 0.05$) PD and LD when compared to GPF pigs.

In the PSC, differences between feeding systems in the SID Lys intake were observed in the second and third week ($P < 0.05$). Consequently, IPF pigs ingested 25% and 32% less SID Lys than GPF, respectively ($P < 0.05$). Similarly to the GSC, the ADG of GPF and IPF pigs allotted in the PSC differed in the third week ($P < 0.05$). During the third week, the ADG of GPF pigs was 45% greater than IPF pigs ($P < 0.05$). The ADFI tended to be lower ($P = 0.09$) for IPF pigs compared to GPF pigs, but it was not affected by the AA ratios ($P > 0.05$). No differences ($P < 0.05$) were observed between FS for G:F over the weeks.

Table 2. Growth performance and body composition of pigs raised in good or poor sanitary conditions and fed with control (CN) or functional amino acid supplemented (AA+) diets within group-phase (GPF) or individual precision feeding (IPF) systems[1].

	GPF		IPF			*P*-value						
Item	CN	AA+	CN	AA+	SEM	FS	Diet	T	FS × Diet	FS × T	Diet × T	FS × Diet × T
Good sanitary conditions												
Initial conditions												
BW, kg	23.22	23.10	23.24	23.30	0.377	0.49	0.84		0.55			
Body protein, kg	2.95	2.94	2.89	3.00	0.055	0.99	0.68		0.59			
Body lipid, kg	4.44	4.60	4.51	4.62	0.039	0.62	0.09		0.75			
Final conditions												
BW, kg	43.43	42.99	38.80	41.00	0.975	0.07	0.23	0.01	0.20	0.01	0.08	**0.05**
Body protein, kg	6.13	6.31	5.50	5.81	0.186	0.09	0.27	0.01	0.49	**0.01**	0.16	0.47
Body lipid, kg	6.24	5.93	6.30	6.12	0.108	0.34	0.56	0.01	0.94	0.28	0.41	0.65
ADG, g/d	722 ^{Aa}	711 ^{Aa}	552 ^{Ab}	636 ^{Aa}	16.37	0.01	0.01	0.01	**0.01**	**0.01**	0.75	0.57
ADFI, g/d	1303	1326	1257	1337	24.16	0.67	0.12	0.01	0.36	0.20	0.21	0.32
G:F, g/g	0.55 ^{Aa}	0.54 ^{Aa}	0.44 ^{Ab}	0.48 ^{Aa}	0.009	0.01	0.47	0.01	**0.03**	**0.03**	0.99	0.78
PD, g/d	114	120	91	102	3.002	**0.01**	**0.05**		0.59			
LD, g/d	61	50	63	58	2.672	0.34	0.16		0.57			
Poor sanitary conditions												
Initial conditions												
BW, kg	23.01	22.81	22.81	23.09	0.368	0.83	0.82		0.19			
Body protein, kg	2.98	2.96	2.90	3.02	0.056	0.89	0.68		0.53			
Body lipid, kg	4.45	4.42	4.47	4.52	0.034	0.40	0.89		0.57			
Final conditions												
BW, kg	38.59	37.22	33.05	35.61	0.751	0.09	0.75	0.01	0.27	**0.01**	0.57	0.14
Body protein, kg	5.56	5.65	4.72	4.80	0.160	0.03	0.73	0.01	0.50	**0.01**	0.77	0.66
Body lipid, kg	5.42	5.58	5.20	5.31	0.067	0.51	0.58	0.01	0.57	**0.03**	0.28	0.90
ADG, g/d	554	515	367	447	19.73	0.01	0.60	0.01	0.11	**0.01**	0.72	0.88
ADFI, g/d	1112	1036	972	1009	24.94	0.09	0.91	0.01	0.25	0.41	0.71	0.84
G:F, g/g	0.49	0.49	0.37	0.43	0.022	0.07	0.25	0.01	0.24	0.35	0.57	0.77
PD, g/d	92	96	63	66	5.091	**0.01**	0.70		0.98			
LD, g/d	34	40	26	30	1.898	**0.02**	0.19		0.78			

SEM = standard error of means, BW = body weight, ADG = average daily gain, ADFI = average daily feed intake, G:F = feed efficiency, PD = protein deposition, LD = lipid deposition.

[1]Data were analyzed considering feeding systems (FS), diets (Diet), time (T) and their interactions as fixed effects. Time was not included as fixed effect for the initial conditions analysis and PD and LD.

$^{Aa-Bb}$Different upper and lowercase superscripts indicate differences ($P < 0.05$) by the F test between Diets in the same FS and FS in the same Diet, respectively.

Values are least square means.

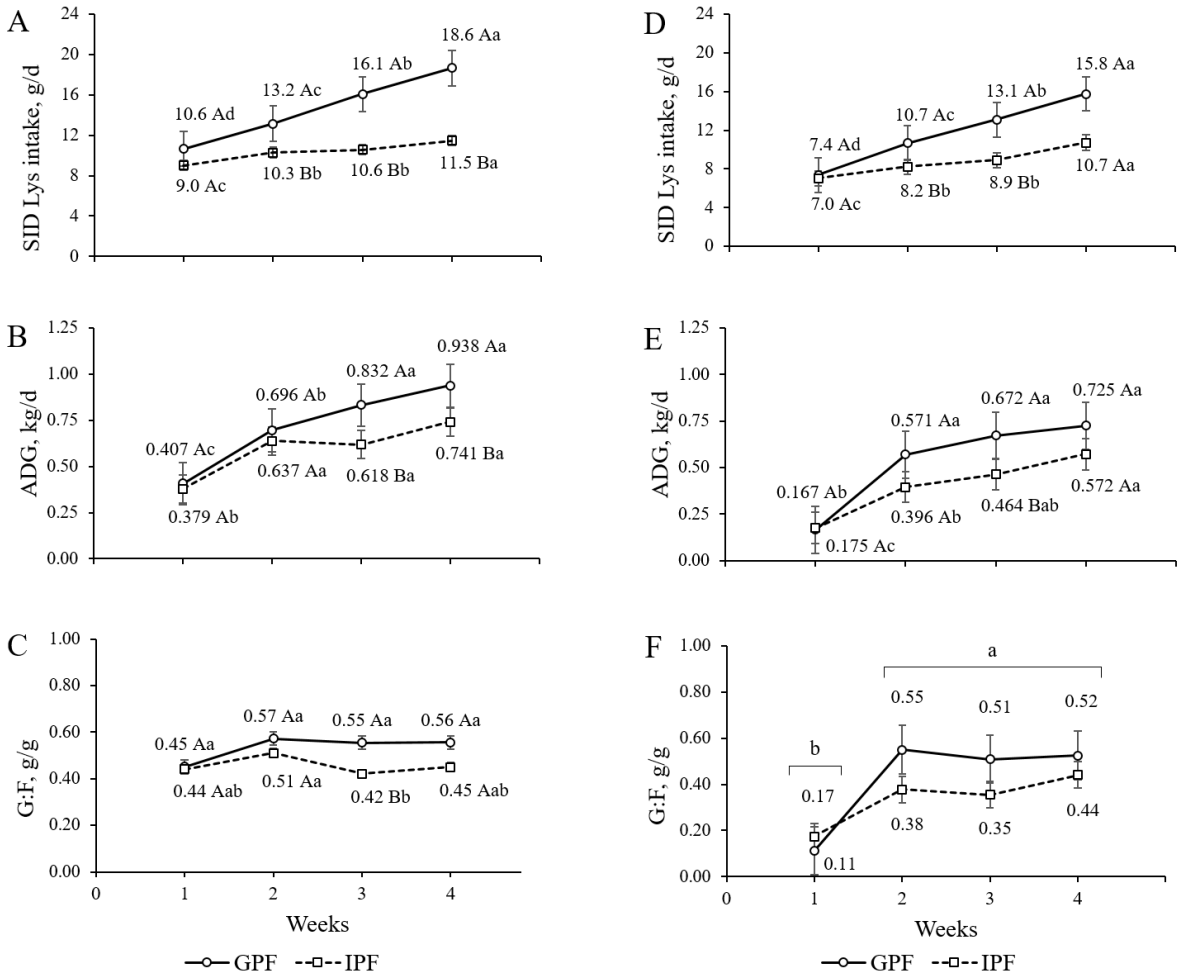

Figure 4. Average SID Lys intake, ADG and G:F of pigs fed with group-phase (GPF) or individual precision feeding (IPF) systems and raised in good (A, B and C) or poor (D, E and F) sanitary conditions. The good sanitary condition was achieved with biosecurity protocols whereas, in the poor condition, pigs were orally inoculated with *Salmonella* Typhimurium and manure from a commercial swine farm was spread on the pen floor. [Aa-Cc]Upper a lowercase letters indicate differences ($P < 0.05$) between feeding systems in each week and weeks in each feeding system by the Tukey test with a 95% interval of confidence. Values are least square means.

3.4 Lysine and nitrogen balance

3.4.1 Good sanitary conditions

The IPF reduced ($P < 0.05$) the SID Lys and N intake and excretion (Table 3) in pigs raised in GSC. Indeed, GPF pigs ingested 38% and 30% more ($P < 0.05$) SID Lys and CP than pigs fed with IPF, respectively. Additionally, GPF excreted 44% more ($P < 0.05$) N than IPF. Consequently, the SID Lys and N efficiencies were higher ($P < 0.05$) in IPF pigs.

3.4.2 Poor sanitary conditions

In the PSC, pigs fed with GPF ingested 41% and 35% more ($P < 0.05$) SID Lys and CP than IPF pigs, respectively. However, differently from the observed in the GSC, the IPF did not improve ($P > 0.05$) the Lys and N efficiencies.

Table 3. Lysine and nitrogen balance of pigs raised in good or poor sanitary conditions and fed with control (CN) or functional amino acid supplemented (AA+) diets within group-phase (GPF) or individual precision feeding (IPF) systems[1].

	GPF		IPF			*P*-value		
Item	CN	AA+	CN	AA+	SEM	FS	Diet	FS × Diet
Good sanitary conditions								
CP intake, g/d	205.4	222.3	157.0	171.1	5.970	**0.01**	**0.09**	0.87
N excretion, g/d	5.72	6.39	4.23	4.16	0.223	**0.01**	0.27	0.36
N efficiency, %	55.6	54.4	57.4	60.4	0.776	**0.01**	0.52	0.16
Lys intake, g/d	13.6	14.2	9.6	10.6	0.418	**0.01**	0.13	0.68
$_{k}$Lys, %[2]	61.9	62.7	70.9	72.9	1.148	**0.01**	0.42	0.74
Poor sanitary conditions								
CP intake, g/d	182.7	175.9	129.5	135.4	5.855	**0.01**	0.96	0.49
N excretion, g/d	5.34	5.00	4.33	4.28	0.162	**0.01**	0.52	0.62
N efficiency, %	51.8	54.0	45.1	48.2	1.899	0.11	0.49	0.91
Lys intake, g/d	12.1	11.3	8.1	8.5	0.395	**0.01**	0.69	0.30
$_{k}$Lys, %[2]	57.8	62.5	54.0	57.7	2.200	0.35	0.36	0.91

SEM = standard error of means;

[1]Data were analyzed considering feeding systems (FS), diets (Diet) and their interactions as fixed effects.

[2]Standardized ileal digestible lysine utilization efficiency ($_k$Lys) = (PD × 0.07) / (SID Lys intake – ((0.313 g Lys/kg DM × ADFI) + (0.0045 g Lys/kg $BW^{0.75}$ × $BW^{0.75}$) + (0.0239 g Lys/kg $BW^{0.75}$ × $BW^{0.75}$))) × 100, where PD is protein deposition, DM is dry matter, ADFI is average daily feed intake and $BW^{0.75}$ is the metabolic body weight.

Lys and N intake and efficiency analysis were performed using only the pigs that were scanned in the DXA (10 pigs/treatment).

3.5 Blood parameters

3.5.1 Good sanitary conditions

No effect of dietary treatments or interactions between feeding systems, diet and time were observed ($P > 0.05$) for any blood parameter in both SC (Table 4). Haptoglobin and triglycerides blood concentrations in GSC pigs decreased on d 7 and remained low until the end of the trial regardless of FS and diet ($P < 0.05$). Similarly, IgA concentration reduced on d 28 ($P < 0.05$) in GSC pigs. Creatinine blood concentration increased on d 7 and remained high until d 28 ($P < 0.05$) in GSC pigs. Feeding system effect was observed for creatinine concentration, and IPF pigs had lower creatinine blood concentration ($P < 0.05$) compared to GPF pigs.

3.5.2 Poor sanitary conditions

In the PSC, we observed a significant time effect for haptoglobin, which increased ($P < 0.05$) on d 7 and remained high ($P < 0.05$) until d 28. Regardless of FS and diet, PSC pigs exhibited a linear increase over time ($P < 0.05$) in the IgG serum concentration, reaching its highest values on d 28. Main effects of time were also observed for IgA, urea and creatinine regardless of FS and diets ($P < 0.05$). All these three parameters exhibited a

quadratic pattern over time with elevated values observed on d 7 ($P < 0.05$) followed by a decrease on d 28 ($P < 0.05$). On the other hand, we noticed a time effect for the triglycerides serum concentration, which decreased ($P < 0.05$) on d 28 for PSC pigs. Interestingly, IPF pigs elicited higher ($P < 0.05$) IgA serum concentration independently of the sampling day or dietary treatment. Similarly to the GSC, PSC pigs within IPF had lower ($P < 0.05$) creatinine when compared to GPF regardless of the sampling time.

Table 4. Blood parameters of pigs raised in either a good or poor sanitary condition and fed with a control (CN) or functional amino acid supplemented (AA+) diet within group-phase (GPF) or individual precision feeding (IPF) systems[1].

	FS		Diet		Days				*P*- value						
Item	GPF	IPF	CN	AA+	0	7	28	SEM	FS	Diet	T	FS × Diet	FS × T	Diet × T	FS × Diet × T
Good sanitary conditions															
Haptoglobin, g/L	0.43	0.41	0.39	0.45	0.49 ^{A}	0.40 ^{B}	0.37 ^{B}	0.021	0.64	0.33	**0.02**	0.72	0.09	0.76	0.56
Albumin, g/dL	3.11	3.00	3.08	3.03	3.06	3.09	3.01	0.030	0.18	0.60	0.38	0.62	0.29	0.06	0.28
IgG, g/dL	0.89	0.94	0.91	0.93	0.90	0.89	0.96	0.020	0.35	0.70	0.21	0.58	0.71	0.31	0.87
IgA, g/dL	0.12	0.13	0.13	0.12	0.14 ^{A}	0.13 ^{A}	0.11 ^{B}	0.003	0.33	0.63	**0.01**	0.78	0.09	0.71	0.93
Urea, mg/dL	15.4	12.9	13.5	14.8	13.8	14.8	14.0	0.564	0.08	0.38	0.62	0.37	0.11	0.67	0.31
Creatinine, mg/dL	1.43	1.29	1.38	1.34	1.24 ^{B}	1.42 ^{A}	1.42 ^{A}	0.018	**0.01**	0.34	**0.01**	0.57	0.87	0.43	0.48
Triglycerides, mg/dL	39.8	43.5	41.4	41.9	53.6 ^{A}	37.7 ^{B}	33.7 ^{B}	1.842	0.35	0.88	**0.01**	0.95	0.67	0.75	0.23
Poor sanitary conditions															
Haptoglobin, g/L	0.91	1.14	1.02	1.03	0.52 ^{B}	1.29 ^{A}	1.27 ^{A}	0.065	0.10	0.93	**0.01**	0.27	0.38	0.92	0.43
Albumin, g/dL	2.96	3.02	3.00	2.98	2.97	2.98	3.01	0.038	0.51	0.87	0.92	0.13	0.89	0.71	0.76
IgG, g/dL	1.15	1.26	1.21	1.21	1.01 ^{C}	1.19 ^{B}	1.42 ^{A}	0.043	0.30	1.00	**0.01**	0.46	0.64	0.67	0.64
IgA, g/dL	0.11	0.14	0.13	0.12	0.13 ^{B}	0.15 ^{A}	0.10 ^{C}	0.004	**0.01**	0.70	**0.01**	0.50	0.09	0.58	0.95
Urea, mg/dL	16.5	15.8	17.0	15.3	13.6 ^{B}	20.0 ^{A}	15.2 ^{B}	0.602	0.61	0.26	**0.01**	0.83	0.69	0.10	0.90
Creatinine, mg/dL	1.45	1.22	1.32	1.36	1.27 ^{B}	1.42 ^{A}	1.32 ^{B}	0.017	**0.01**	0.24	**0.01**	0.31	0.68	0.71	0.25
Triglycerides, mg/dL	39.8	51.0	46.9	43.9	52.0 ^{A}	46.1 ^{A}	38.2 ^{B}	2.431	0.06	0.60	**0.04**	0.99	0.20	0.23	0.55

SEM = standard error of means.

[1]Data were analyzed considering feeding systems (FS), diets (Diet), time (T) and their interactions as fixed effects.

$^{A-C}$Within a row different superscripts indicate differences ($P < 0.05$) between sampling days by multiple comparison Tukey test with a 95% interval of confidence.

Values are least square means.

4. Discussion

4.1 Response of pigs to the sanitary conditions

The sanitary challenge model used in the PSC aimed to replicate on-farm conditions in which pigs are infected with an oral-fecal pathogen in an environment with poor cleaning practices. Even though we did not perform statistical analysis to compare the SC, as the animals were in two different buildings, the different patterns in the analyzed variables illustrates a difference in the health status between conditions. The effectiveness of the *Salmonella* Typhimurium inoculation was validated by the high number of positive pigs to *Salmonella* Typhimurium and high *Salmonella* Typhimurium shedding on d 3.

As expected, we observed an increase in the rectal temperature within 24 h after introducing the pigs to the PSC. Similar increases in rectal temperature were also observed by authors who used a similar sanitary challenge approach (Rodrigues et al., 2021; Valini et al., 2023). Fever is a physiological response that occurs during systemic immune activation to transform the organism in a hostile environment to pathogen proliferation (Sandberg et al., 2007). The decrease in the *Salmonella* detection and return of the rectal temperature, IgA, urea and creatinine to the basal values on d 28 indicates that the immune activation was more pronounced in the beginning of the trial. However, pigs in the PSC showed an increase of 250% in haptoglobin concentration on d 7 and 246% on d 28 when compared to d 0. Haptoglobin is synthesized in the presence of pro-inflammatory cytokines and it is considered a blood indicator of health status in pigs (Johnson, 1997; Le Floc'h et

al., 2009). Thus indicating that even with the smaller immune activation after the first week of challenge, pigs in PSC seemed to be challenged overall the experimental period.

Increased IgG and IgA concentrations in PSC pigs indicates that the SC was able to induce a humoral immune response. Differently from IgG, the IgA concentration decreased on day 28. IgA is synthesized in mucosa-associated lymphoid tissues in the presence of pathogens (Pereira and Woof, 2019). Therefore, the decrease in the number of positive pigs to *Salmonella* Typhimurium and *Salmonella* Typhimurium shedding may have reduced the stimulus to its synthesis. Additionally, the IgG half-life in the blood is higher than IgA (Schroeder et al., 2010; Pereira and Woof, 2019) which might have contributed to the early reduction in the IgA concentration.

The immune system activation increases the mobilization of body reserves (e.g., muscle and adipose tissues) to provide nutrients to support the immune response (Campos et al., 2017; Le Floc'h et al., 2018). However, muscular AA profile differs from the AA profile required by the immune system, inducing an AA imbalance that increases AA oxidation and urea synthesis (Reeds et al., 1994). Even though we did not directly measured muscle proteolysis, the observed changes in creatinine and urea serum concentrations over time are indicators of muscle breakdown and AA oxidation. Creatinine is a final metabolism product of the muscle and its serum concentration is associated with muscle proteolysis (Yin et al., 2017). The use of dietary AA and body reserves to assemble the immune response impair PD and N retention (Barcellos et al., 2021; Valini et al., 2023). In this trial, although we have not compared SC, we noticed that PSC pigs exhibited numerically lower PD, N and SID Lys efficiencies compared to GSC pigs. Thus, potentially supporting the idea that dietary energy and nutrients are preferentially used by

the immune system and that the unused ones can be used for growth or other productive functions.

On the other hand, despite the evidence of immune system activation, serum creatinine concentration also increased over time in pigs within GSC. Additionally, an elevated creatinine concentration was observed in pigs fed within GPF system. Pigs in GPF had greater final body protein mass and PD during the trial regardless of the SC. Thus, the changes in their serum creatinine alone are likely associated with the increase in the body protein mass (Remus et al., 2019) rather than proteolysis to supply AA to the immune system.

It was previously demonstrated that an immune challenge increases the energy requirement for maintenance (Huntley et al., 2018). Similarly to the skeletal muscle, immune system activation promotes lipolysis while inhibiting lipogenesis, thereby increasing the availability of fatty acids that can be used as energy source (Guo et al., 2015; Valini et al., 2023). Although the triglycerides concentration reduced on d 28 in both SC, on d 7, pigs in PSC exhibited an increased triglycerides concentration. As the highest values of *Salmonella* shedding and fever were found in the first week, the increased in triglycerides could indicate a greater energy demand during this period due its toll in the immune response. Altogether, these results indicate a successfully immune system activation of pigs in PSC.

4.2 Response of pigs to different feeding systems

The mathematical model developed to estimate individual nutrient requirements by Hauschild et al. (2012) was validated and tested in different trials (Andretta et al., 2014;

Cloutier et al., 2015; Andretta et al., 2016), consistently demonstrating its ability to reduce the excess of nutrients provided while sustaining growth performance (Santos et al., 2018; Remus et al., 2019). Therefore, we hypothesized that IPF would be an alternative feeding strategy that could be used for challenged pigs once the immune system activation increases the population variability (van der Peet et al., 2019; Ortiz et al., 2024).

Indeed, we observed a reduction in the intake and higher efficiency of utilization of N and SID Lys of pigs in GSC within IPF system. However, in this experiment, IPF impaired the growth performance in both SC. Interestingly, the ADFI of GPF and IPF did not significantly differ in GSC and PSC. These findings indicate that the main reason underlying the difference between both feeding systems was the dietary nutrient composition. Accordingly, the BW, ADG and G:F of GSC pigs within IPF increased when they were fed with AA+ diets, suggesting that the IPF diets were AA deficient. Indeed, the IPF pigs' performance increased when fed more AA without changing the Lys supply. These findings are aligned with those observed by Remus et al. (2019a,b) whose demonstrated that, within IPF, pigs have a linear response when fed with 30% more Thr and Met:Lys. Thus, showing the need of adjusting AA ratios in IPF fed pigs before it could be used in conditions different from optimal experimental settings.

Compared to GSC, we observed a decrease in the ADFI for pigs in PSC in the first experimental week which could be attributed to the immune system activation (Johnson, 1997). However, we did not expect to observe a similar but less pronounced decrease in ADFI for pigs in GSC. Since no evidence of immune system activation was noticed, we assume that the decrease in ADFI in GSC pigs was associated with feed changes from adaptation to the experimental period and the stress related to the number of experimental

procedures performed at the beginning of the trial. The differences in the ADG between GPF and IPF pigs during the trial were more pronounced in the PSC (24%) than in the GSC (17%). Conversely, the ADG of IPF and GPF were similar in the last week of trial in the PSC. This suggests that, apart from the ADG reduction in the first week, another factor might have influenced the performance of PSC pigs fed with IPF. During the immune activation, reduction in the ADG often occurs due to the redirection of nutrients from growth towards the immune system. The amount of nutrients required for the immune responses depends on the challenge imposed (e.g., LPS, poor hygiene, pathogen inoculation), pathogen (e.g., virulence, concentration) and individual responses (Sandberg et al., 2007). These factors are not accounted for in the current IPF model. Therefore, IPF pigs raised in the PSC were fed with diets that met their requirements for growth without considering the metabolic changes relate to the immune system activation. Hence, IPF pigs may have to rely on mobilizing more body reserves to support their immune response. Indeed, IPF significantly reduced growth performance, PD and LD of PSC pigs when compared to GPF. The absence of statistical difference of ADG in the last week may have occurred due to the resolution of the immune system activation that increased nutrient availability for growth.

Moreover, to account for the variability between individuals, the IPF model forecasts a 1-d prediction of DFI and 7-d prediction of BW until new real-time data is acquired (Hauschild et al., 2012). This data is used to determine the required individual dietary SID Lys concentration. However, data collected during periods of growth disruption (e.g., health challenges, handling procedures) are also used as input to predict future growth performance. Such changes in growth directly affect the empirical model

estimations for DFI and DG, leading to a likely underestimation of SID Lys requirements of IPF in both GSC and PSC. Thus explaining the lowered growth performance in GSC as well when compared to GPF. The error is exacerbated by the fact that feed intake is captured daily, while BW is captured weekly due the lack of reliable real-time weight measures. This temporal misalignment in the two primary variables hinders the accuracy of predictions. Although the recovery of DFI can be observed on the day-to-day basis, the DG, which is the main driver of SID Lys requirements in the model, will only be available 7 days later. Thus, impairing the ability to short-term correct SID Lys predictions when the animals recover their regular performance after a disturbance. This limitation is evidenced by the higher ADG and BW of IPF pigs that received AA+. These findings highlight the need for robust real-time data collection and the need of improvements in the empirical component of the model for practical use (Hauschild et al., 2020). It was the first time that the IPF model was used in a drastic sanitary scenario out of optimal conditions. The model has a smoothing component used to minimize the influence of potential drastic changes of BW and ADFI on AA estimation (Hauschild et al., 2012). The results observed herein indicate that the smoothing component used in previous trials (Andretta et al., 2014; Santos et al., 2018; Remus et al., 2019) is not adequate when a high magnitude BW and ADFI fluctuations are encountered. Therefore, care should be taken when applying the current IPF model for pig populations subjected to sanitary or other challenges changing feed intake patterns or the animal performance.

4.3 Effect of functional amino acid supplementation

It was previously shown that the immune system activation modifies the AA requirements of pigs (Jayaraman et al., 2015; Jayaraman et al., 2017). Such modifications are related to the fact that pigs require AA to build the immune response (Reeds et al., 1994; van der Peet et al., 2019). Additionally, extra supply of FAA as Met, Thr and Trp are used in specific metabolic functions that allow pigs to cope with the negative effects of the immune system activation (Le Floc'h et al., 2018; Rodrigues et al., 2021; Valini et al., 2023).

For instance, Met is a precursor of glutathione peroxidase, an antioxidant enzyme that neutralizes reactive oxygen species produced during the immune challenge (Le Floc'h et al., 2018). Threonine is the major component of mucin that prevents microrganism translocation from the intestinal lumen to the bloodstream (Koo et al., 2020). Finally, Trp down regulates the T-cells proliferation and prevents an exacerbated immune response (Le Floc'h et al., 2018). In this sense, we expected that the combined supplementation of Met + Cys, Thr and Trp above their optimal ratio for growth would alleviate the negative effects of the immune system activation as observe by other authors (van der Meer et al., 2016; Rodrigues et al., 2021; Valini et al., 2023). However, in this experiment, feeding pigs with FAA did not influence blood parameters or performance variables in the PSC.

Furthermore, most of the studies which evaluated the combined effect of increasing 20% of the Met + Cys, Thr and Trp to Lys ratio, used the AA ratios recommended by the NRC (van der Meer et al., 2016; Rodrigues et al., 2021; Valini et al., 2023). To the best of our knowledge, this was the first experiment which evaluated the supplementation of these FAA using diets formulated with the InraPorc ratios (van Milgen et al., 2008). The InraPorc ratios of Met + Cys, Thr and Trp to Lys are 7%, 8% and 15% higher than the values of

NRC (2012), respectively. Therefore, the absence of FAA supplementation effect in this study might be related to the fact that the CN diet was already providing the FAA to support the immune response.

To create the PSC, we used the protocol developed by Valini et al. (2023). Differently from the data observed by these authors, the haptoglobin concentration analyzed herein remained elevated until the end of the trial. Furthermore, the ADG in the first week of challenge reduced drastically in the PSC. This suggests that the pigs' immune system was highly stimulated during this period. The intensity of the immune activation influences the maintenance requirements and the rate of which AA are required for the synthesis of immune components (Pastorelli et al., 2012; van der Peet et al., 2019). As an example, in a meta-analysis performed by Pastorelli et al. (2012), the authors demonstrated that the changes in maintenance account for 74% of the reduction in growth performance caused by digestive bacterial infections. However, the same authors showed that this percentage is reduced to 30% in respiratory diseases. Thus, even when FAA are supplemented, the intensity of the challenge may hide its benefits on the growth performance due to its redirection for the synthesis of other proteins than those aforementioned.

Finally, the immune response is a dynamic process with feedback mechanisms that enhance activation in the presence of an antigen and decrease activation once the antigen is neutralized (Johnson, 1997). The rectal temperature, positive pigs for *Salmonella* shedding, and blood parameters observed herein confirm that the acute phase of the sanitary challenge occurred in the first experimental week. During this period, GPF pigs fed either AA+ or CN diets consumed similar amounts of FAA. The similarity in FAA

intake between CN and AA+ pigs can be attributed to the lower ADFI of CN pigs in the first week of challenge. Pigs have different abilities to cope with the sanitary challenge. Therefore, even when subjected to infections with the same pathogen, pigs exhibit different degrees of feed intake reduction (Sandberg et al., 2007; Ortiz et al., 2024). As a result, CN and AA+ pigs had similar amounts of FAA available to synthesize immune system components during the acute phase of the sanitary challenge. Such results may have prevented better immune protection in AA+ pigs, resulting in no significant difference compared to the CN group. Thus, our findings suggest that the benefits of FAA for immune-challenged pigs may depend on the AA ratios used to formulate the diets, the intensity of the immune activation, or the AA intake during the challenge.

5. Conclusions

In conclusion, the combined effect of precision feeding and increased ratios of AA to Lys ratios did not alleviate the negative impacts of the immune system activation in growing pigs nor increase their nutrient efficiency. Increasing the AA ratios of Met + Cys from 0.60 to 0.72, Thr from 0.65 to 0.78, and Trp from 0.20 to 0.24 might not have reduced the immune activation due to the AA ratios used in the diets, the intensity of the immune response, or the amount of AA intake during the sanitary challenge. Large fluctuations in individual BW and DFI hinder the empirical model's ability to correctly forecast these variables, resulting in poor estimations of SID Lys requirement in the IPF system. This study highlights the need to take into account the utilization of nutrients by the immune system when determining the nutritional requirements of animals. Although complex and poorly understood, nutrient utilization by the immune system may significantly penalize

the pig, especially when the excess of dietary nutrients are minimized. Despite the complexity, these requirements must be included in our models when they are to be used in commercial farms, particularly when using precision feeding systems.

Conflict of Interest Statement

H. G. B. and J. K. H. are employees of Evonik Operations GmbH and Evonik Brasil Ltda, respectively, which financed part of this study. The other authors declare no real or perceived conflicts of interest.

Literature Cited

Andretta, I., Pomar, C., Rivest, J., Pomar, J., Lovatto, P. A., & Radünz Neto, J. (2014). The impact of feeding growing–finishing pigs with daily tailored diets using precision feeding techniques on animal performance, nutrient utilization, and body and carcass composition. *Journal of Animal science*, *92*(9), 3925-3936.

Andretta, I., Pomar, C., Kipper, M., Hauschild, L., & Rivest, J. (2016). Feeding behavior of growing–finishing pigs reared under precision feeding strategies. *Journal of animal science*, *94*(7), 3042-3050.

AOAC, Official Methods of the Association of Official Analytical Chemists. (2010). AOAC official method 994.12: amino acids in feeds.

Barcellos, J., Alves, W. J., Arnaut, P. R., Fonseca, L., Muniz, J. C. L., Dorigam, J. C. D. P., ... & Hannas, M. I. (2021). Assessment of digestible lysine requirements in lipopolysaccharide-challenged pigs. *Journal of animal science*, *99*(12), skab336.

Campos, P. H. R. F., Floc'h, L., Noblet, J., & Renaudeau, D. (2017). Physiological responses of growing pigs to high ambient temperature and/or inflammatory challenges. *Revista Brasileira de Zootecnia*, *46*, 537-544.

Cloutier, L., Pomar, C., Montminy, M. L., Bernier, J. F., & Pomar, J. (2015). Evaluation of a method estimating real-time individual lysine requirements in two lines of growing–finishing pigs. *Animal*, *9*(4), 561-568.

de Lange, C. F. M., Morel, P. C. H., & Birkett, S. H. (2003). Modeling chemical and physical body composition of the growing pig. *Journal of Animal Science*, *81*(14_suppl_2), E159-E165.

Goodband, B., Tokach, M., Dritz, S., DeRouchey, J., & Woodworth, J. (2014). Practical starter pig amino acid requirements in relation to immunity, gut health and growth performance. *Journal of Animal Science and Biotechnology*, *5*(1), 1-11.

Guo, J., Liu, Z., Sun, H., Huang, Y., Albrecht, E., Zhao, R., & Yang, X. (2015). Lipopolysaccharide challenge significantly influences lipid metabolism and proteome of white adipose tissue in growing pigs. *Lipids in Health and Disease*, *14*, 1-11.

Hauschild, L., Pomar, C., & Lovatto, P. A. (2010). Systematic comparison of the empirical and factorial methods used to estimate the nutrient requirements of growing pigs. *Animal*, *4*(5), 714-723.

Hauschild, L., Lovatto, P. A., Pomar, J., & Pomar, C. (2012). Development of sustainable precision farming systems for swine: estimating real-time individual amino acid requirements in growing-finishing pigs. *Journal of Animal Science*, *90*(7), 2255-2263.

Hauschild, L., Kristensen, A. R., Andretta, I., Remus, A., Santos, L. S., & Pomar, C. (2020). Toward better estimates of the real-time individual amino acid requirements of growing-finishing pigs showing deviations from their typical feeding patterns. *animal*, *14*(S2), s371-s381.

Huntley, N. F., Nyachoti, C. M., & Patience, J. F. (2018). Lipopolysaccharide immune stimulation but not β-mannanase supplementation affects maintenance energy requirements in young weaned pigs. *Journal of animal science and biotechnology*, *9*, 1-16.

Jayaraman, B., Htoo, J., & Nyachoti, C. M. (2015). Effects of dietary threonine: lysine ratios and sanitary conditions on performance, plasma urea nitrogen, plasma-free threonine and lysine of weaned pigs. *Animal Nutrition*, *1*(4), 283-288.

Jayaraman, B., Htoo, J. K., & Nyachoti, C. M. (2017). Effects of different dietary tryptophan: lysine ratios and sanitary conditions on growth performance, plasma urea nitrogen, serum haptoglobin and ileal histomorphology of weaned pigs. *Animal Science Journal*, *88*(5), 763-771.

Johnson, R. W. (1997). Inhibition of growth by pro-inflammatory cytokines: an integrated view. *Journal of animal science*, *75*(5), 1244-1255.

Koo, B., Choi, J., Yang, C., & Nyachoti, C. M. (2020). Diet complexity and l-threonine supplementation: effects on growth performance, immune response, intestinal barrier function, and microbial metabolites in nursery pigs. *Journal of Animal Science*, *98*(5), skaa125.

Le Floc'h, N., Lebellego, L., Matte, J. J., Melchior, D., & Sève, B. (2009). The effect of sanitary status degradation and dietary tryptophan content on growth rate and tryptophan metabolism in weaning pigs. *Journal of Animal Science*, *87*(5), 1686-1694.

Le Floc'h, N., Wessels, A., Corrent, E., Wu, G., & Bosi, P. (2018). The relevance of functional amino acids to support the health of growing pigs. *Animal Feed Science and Technology*, *245*, 104-116.

Mahan, D. C., & Shields Jr, R. G. (1998). Essential and nonessential amino acid composition of pigs from birth to 145 kilograms of body weight, and comparison to other studies. *Journal of Animal Science*, *76*(2), 513-521.

Möhn, S., Gillis, A. M., Moughan, P. J., & De Lange, C. F. M. (2000). Influence of dietary lysine and energy intakes on body protein deposition and lysine utilization in the growing pig. *Journal of Animal Science*, *78*(6), 1510-1519.

National Research Council, Southern, L. L., Adeola, O., & de Lange, C. F. (2012). *Nutrient requirements of swine*. National Academies Press.

Oliveira, L. G., Carvalho, L. F. O. S., Masson, G. C. I. H., & Feliciano, M. A. R. (2010). Infecção experimental por Salmonella enterica subespécie enterica sorotipo Panama e tentativa de transmissão nasonasal em leitões desmamados. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, *62*, 1340-1347.

Ortiz, M. T., Arnaut, P. R., Valini, G. A. C., França, I., Silva, C. A., de Oliveira, M. J. K., ... & Hauschild, L. (2024). A salmonella-challenge impacts the variability of performance, body composition and lysine requirements of growing pigs under poor housing conditions. *Livestock Science*, 105462.

Pastorelli, H., Van Milgen, J., Lovatto, P., & Montagne, L. (2012). Meta-analysis of feed intake and growth responses of growing pigs after a sanitary challenge. *Animal*, *6*(6), 952-961.

Pereira, P S.., & Woof, J. M. (2019). IgA: structure, function, and develop ability. *Antibodies*, *8*(4), 57.

Pomar, C., & Rivest, J. (1996, July). The effect of body position and data analysis on the estimation of body composition of pigs by dual energy X-ray absorptiometry (DEXA). In *Proceedings of the 46th Annual Conference of the Canadian Society of Animal Science, Lethbridge, AB, Canada* (p. 26).

Pomar, C., Hauschild, L., Zhang, G. H., Pomar, J., & Lovatto, P. A. (2011). Precision feeding can significantly reduce feeding cost and nutrient excretion in growing

animals. In *Modelling nutrient digestion and utilisation in farm animals* (pp. 327-334). Wageningen Academic Publishers, Wageningen.

Rakhshandeh, A., & De Lange, C. F. M. (2011). Immune system stimulation in the pig: effect on performance and implications for amino acid nutrition. *Manipulating Pig Production XIII. Werribee: Australasian Pig Science Association*, *31*, 46.

Reeds, P. J., Fjeld, C. R., & Jahoor, F. (1994). Do the differences between the amino acid compositions of acute-phase and muscle proteins have a bearing on nitrogen loss in traumatic states?. *The Journal of nutrition*, *124*(6), 906-910.

Remus, A., Hauschild, L., Corrent, E., Létourneau-Montminy, M. P., & Pomar, C. (2019a). Pigs receiving daily tailored diets using precision-feeding techniques have different threonine requirements than pigs fed in conventional phase-feeding systems. *Journal of animal science and biotechnology*, *10*, 1-17.

Remus, A., Pomar, C., Perondi, D., Gobi, J. P., da Silva, W. C., de Souza, L. J., & Hauschild, L. (2019b). Response to dietary methionine supply of growing pigs fed daily tailored diets or fed according to a conventional phase feeding system. *Livestock Science*, *222*, 7-13.

Remus, A., Hauschild, L., & Pomar, C. (2020). Simulated amino acid requirements of growing pigs differ between current factorial methods. *animal*, *14*(4), 725-730.

Rodrigues, L. A., Wellington, M. O., González-Vega, J. C., Htoo, J. K., Van Kessel, A. G., & Columbus, D. A. (2021). Functional amino acid supplementation, regardless of dietary protein content, improves growth performance and immune status of weaned pigs challenged with Salmonella Typhimurium. *Journal of Animal Science*, *99*(2), skaa365.

Sandberg, F. B., Emmans, G. C., & Kyriazakis, I. (2006). A model for predicting feed intake of growing animals during exposure to pathogens. *Journal of animal science*, *84*(6), 1552-1566.

Sandberg, F. B., Emmans, G. C., & Kyriazakis, I. (2007). The effects of pathogen challenges on the performance of naïve and immune animals: the problem of prediction. *Animal*, *1*(1), 67-86.

Santos, L. S. D., Pomar, C., Campos, P. H. R. F., da Silva, W. C., Gobi, J. D. P., Veira, A. M., ... & Hauschild, L. (2018). Precision feeding strategy for growing pigs under heat stress conditions. *Journal of Animal Science*, *96*(11), 4789-4801.

Schroeder Jr, H. W., & Cavacini, L. (2010). Structure and function of immunoglobulins. *Journal of allergy and clinical immunology*, *125*(2), S41-S52.

Valini, G. A. C., Righetti Arnaut, P., França, I., Trevisan Ortiz, M., Karpeggiane de Oliveira, M. J., Brandão Melo, A. D., ... & Hauschild, L. (2023). Increased dietary Trp, Thr, and Met supplementation improves growth performance and protein deposition of salmonella-challenged growing pigs under poor housing conditions. *Journal of Animal Science*, *101*, skad141.

van der Meer, Y., Lammers, A., Jansman, A. J. M., Rijnen, M. M. J. A., Hendriks, W. H., & Gerrits, W. J. J. (2016). Performance of pigs kept under different sanitary conditions affected by protein intake and amino acid supplementation. *Journal of Animal Science*, *94*(11), 4704-4719.

van der Peet-Schwering, C. M. C., Koopmans, S. J., & Jansman, A. J. M. (2019). Amino acid requirements in relation to health status in growing and finishing pigs (No. 1168). Wageningen Livestock Research.

van Milgen, J., Valancogne, A., Dubois, S., Dourmad, J. Y., Sève, B., & Noblet, J. (2008). InraPorc: a model and decision support tool for the nutrition of growing pigs. *Animal Feed Science and Technology*, *143*(1-4), 387-405.

Weber, K., & Osborn, M. (1969). The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. *Journal of Biological Chemistry*, *244*(16), 4406-4412.

Wellock, I. J., Emmans, G. C., & Kyriazakis, I. (2004). Modeling the effects of stressors on the performance of populations of pigs. *Journal of Animal Science*, *82*(8), 2442-2450.

Wood, R. L., Rose, R., Coe, N. E., & Ferris, K. E. (1991). Experimental establishment of persistent infection in swine with a zoonotic strain of Salmonella newport. *American journal of veterinary research*, *52*(6), 813-819.

Yin, C., Liu, W., Liu, Z., Huang, Y., Ci, L., Zhao, R., & Yang, X. (2017). Identification of potential serum biomarkers in pigs at early stage after Lipopolysaccharide injection. *Research in veterinary science*, *111*, 140-146.

CAPÍTULO 3 – Increasing the dietary threonine-to-lysine ratio improves the growth performance of slow-growing pigs within group precision feeding.

Este capítulo foi redigido de acordo com as normas da revista **Journal of Animal Science**

Running head: Precision feeding and variability in growth

Increasing the dietary threonine-to-lysine ratio improves the growth performance of slow-growing pigs within group precision feeding.

Pedro Righetti Arnaut[†‡], Candido Pomar[‡], Luciano Hauschild[†], Aline Remus[‡].

*Corresponding author: aline.remus@agr.gc.ca

[†]Department of Animal Science, São Paulo State University (UNESP), School of Agricultural and Veterinary Sciences, Jaboticabal, São Paulo, Brazil, 14884-900.

[‡]Sherbrooke Research and Development Centre, Agriculture and Agri-Food Canada, 2000 Rue College, Sherbrooke, Quebec, Canada, J1M 0C8.

[1]The authors acknowledge the financial support received from the São Paulo Research Foundation (FAPESP, grant numbers 2018/15559-7 and 2019/10843-1), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES – Finance Code 001) and the Brazilian National Council for Scientific and Technological Development (CNPq, grants number 408287/2021-7, and 442675/2023-2). Additionally, we would like to thank the statistician Steve Methot for his assistance with the statistical analysis. Furthermore, we would like to thank the research assistant Sophie Horth for her outstanding contribution in organizing and conducting the trials. The authors also acknowledge the laboratory technician Jean-Philippe Brousseau for his assistance with the blood sampling and laboratory analysis. Finally, we would like to thank Mélanie Turcote and the entire swine

complex team of Agriculture and Agri-Food Canada for their assistance and fantastic professionalism in conducting this trial.

Lay Summary

The current precision feeding model estimates the dietary lysine requirements and assume that the other amino acids are required at conventional proportions to lysine. However, studies have demonstrated a linear increase in the growth performance of pigs when fed with methionine and threonine to lysine ratios up to 130% of those usually used in conventional group-phase feeding. It remains unclear whether the higher amino acid ratios improve the overall population performance or specific categories of pigs within the population. The aim of this study was to evaluate the response of pigs fed graded threonine to lysine ratios within precision feeding and to determine if this response is influenced by the growth rate categories.

Teaser Text

This study emphasizes the variation in nutritional requirements that exists within pig populations and highlights that the use of standard amino acid ratios may not represent the needs of all pigs. By accounting for this variability, growth can be optimized while also increasing nutrient efficiency of the swine production system.

Abstract: Growth response to nutrient supply varies greatly between and within pig populations. We hypothesized that part of the variation in growth rate (Gr) observed in nutritional trials is due to pigs requiring different amino acid ratios among them. Therefore,

this study evaluated the growth response of pigs receiving Thr-to-Lys ratio varying from 80 to 140% of the estimated ideal 0.65 ratio within group precision feeding (GPF), and if this requirement varies within slow, medium and fast Gr categories. Sixty pigs (30 females and 30 barrows) of 38.1 (± 3.8) kg of BW were distributed in complete randomized blocks with a 3 × 5 factorial arrangement. Blocks were used as a fixed experimental factor and consisted of three Gr: fast, medium or slow based on their daily weight gain pre-trial. Dietary treatments consisted of a conventional phase feeding receiving 100% (CPF100) and 4 group-precision feeding (GPF) receiving 80% (GPF80), 100% (GPF100), 120% (GPF120) and 140% (GPF140) of the optimal Thr to Lys ratio of 0.65. Conventional group-phase pigs were fed with a single diet with 0.97% SID Lys, whereas GPF diets were adjusted every two days to meet the SID Lys requirements of the most demanding pig in the pen. Pigs were blood sampled and scanned with dual X-ray to estimate their body chemical composition on the beginning and end of the 21 d trial. A mixed model considering the pig as the experimental unit was used to analyse the data, and polynomial contrasts were used to compare the treatments. The Thr ratios linearly increased ($P \leq 0.05$) the ADG of slow-growing pigs, achieving similar ADG to medium and fast pigs. Cubic effect ($P \leq 0.05$) was observed for the PD:DG, indicating leaner gain on GPF100 and GPF140. Plasma threonine levels increased with higher Thr ratios ($P \leq 0.05$), while lysine levels decreased regardless of Gr ($P \leq 0.05$). Threonine levels and Gr did not influence blood parameters analysed ($P > 0.10$). The results suggest that optimizing Thr ratios can specifically benefit slow-growing pigs, although the traditional ideal protein concept may not fully address the nuances of precision feeding. In conclusion, the Thr-to-Lys ratio that maximize the performance of pigs within GPF varies with Gr. Future research should focus

on refining individual amino acid requirements to better align with the dynamic needs of precision-fed pigs for improved growth and productivity.

Keywords: group homogeneity, growth rate, ideal protein profile, nutrient requirements, precision nutrition

Abbreviations

AA, amino acid

ADG, average daily gain

ADFI, average daily feed intake

BW, body weight

CPF, conventional phase feeding

CRP, C-reactive protein

GPF, group precision feeding

GPx, glutathione peroxidase

LD, lipid deposition

PD, protein deposition

SID, standard ileal digestible

STTD, standard total tract digestible

1. Introduction

Pig production is an important social and economic component for several countries. As pig production increases to meet world demand, other concerns arise, including how to develop sustainable systems (Ominski et al., 2021). Part of sustainability

involves developing feeding programs that minimize environmental impact while increasing producers' economic returns and ensuring that pig needs are met. Conventionally, pigs are fed in groups (**CPF**) with a single diet provided for an extended period of time (e.g. a phase of 21 days) and formulated to maximize population growth performance or minimize feeding costs. Since pigs' nutritional requirements vary over time and between individuals, providing a single diet often results in most pigs receiving nutrients above the requirement and excreting this excess (Hauschild et al., 2010; Andretta et al., 2014; Remus et al., 2019a). In this scenario, precision feeding is a valuable tool designed to estimate individual SID Lys requirements accounting for the requirements variability (Hauschild et al., 2012). Precision feeding can be defined as providing daily tailored diets to animals aiming to provide the right amount of feed, with the right composition at the right time (Pomar et al., 2019). Additionally, precision feeding techniques can be applied to individuals or for a small group of pigs (**GPF**). When compared to CPF, the application of GPF was shown to reduce crude protein, SID Lys, and phosphorus intake by 10%, 17% and 7%, respectively, and to reduce nitrogen excretion by 12% without any losses in growth performance (Andretta et al., 2014). Additionally, feed is responsible for 60% of CO_2 emissions, and precision feeding can help to decrease the environmental impact by 8% compared to conventional phase feeding systems (Llorens et al., 2024).

In precision feeding diets, amino acids (**AA**) are included at fixed proportions with Lys based on the assumption that the optimal AA-to-Lys ratios are the same as those for CPF (Andretta et al., 2016; Cloutier et al., 2014; Santos et al., 2018). However, studies observed a linear response in the ADG of individually precision-fed pigs receiving graded

ratios of Met and Thr-to-Lys ratios (**Thr:Lys**) up to 130% of those used in CPF (Remus et al., 2019b; Remus et al., 2019c). Furthermore, in early-growing pigs, increases in Thr:Lys resulted in improved protein deposition and increases in plasmatic albumin, total protein and C-reactive protein (Remus et al., 2019c). These results suggest that changes in AA profile can improve not only growth performance but also might boost the immune response of pigs.

Still, a large variance in response to the same AA ratio within treatments is often observed in titration studies (Barea et al., 2009, Remus et al., 2019b, c; Soto et al., 2019). Such variation is even larger if a sanitary challenge is present (Cadéro et al., 2020; Ortiz et al., 2024). The reasons for variability in growth are still poorly understood. However, studies demonstrated that the use of a single feed does not maximize population performance because pigs the dietary composition that optimize growth varies between individuals (Aymerich et al., 2020). Therefore, it is possible that differences in growth rate (**Gr**) are linked to differences in AA requirements among pigs. Developing strategies that improve herd homogeneity could improve not only economic but also environmental impacts (Davoudkhani et al., 2020).

We hypothesized that increasing Thr:Lys would optimize the growth performance in GPF pigs compared to a standard CPF system. Additionally, increasing Thr:Lys may improve the growth performance and the concentration of plasmatic proteins especially of slow Gr pigs within GPF. The aim of this study was to evaluate the growth performance of pigs receiving Thr:Lys varying from 80 to 140% of the estimated ideal 0.65 ratio (van Milgen et al., 2008) within GPF, and if this performance varies within slow, medium and fast Gr categories.

2. Material and Methods

The animals were housed and cared for following the Agriculture and Agri-Food Canada guidelines (National Farm Animal Care Council, 2012) and all the experimental procedures performed were previously approved by the Institutional Committee of Animals Protection (protocol nº 617).

2.1 Animal husbandry and experimental design

A total of 60 pigs (30 females and 30 barrows) with the same genotype (Duroc × [Yorkshire × Landrace]) were used in this experiment. The pigs were group-housed in a 43-m^2 pen with concrete slatted floor and nipple drinkers in a mechanically ventilated room. The pigs received an RFID ear tag to give them access to the electronic feeders (IVOG system, Hokofarm Group BV, Emmeloord, NE) in the pen. The feeders were designed to feed pigs individually while recording their daily feed intake (**ADFI**). A 14 d period was used to adapt the pigs to the feeders and collect individual baseline information on BW and ADFI. During this period, the pigs were individually fed with a diet formulated with 1.20% SID Lys and 2,410 kcal/kg of net energy, 0.34% and 0.74% of digestible P and total Ca, respectively.

After the adaptation period, the pigs (38.1 ± 3.8 kg of BW) were distributed in complete randomized blocks with a 3 × 5 factorial arrangement consisting of 3 growth rate groups and 5 dietary treatments. Individual BW during the adaptation period was used to predict the average daily gain (**ADG**) and categorize the pigs as Fast, Medium and Slow Gr. After categorization, pigs (2 females and 2 barrows) with similar Gr were placed in

pens (6-m^2) with concrete slatted floor and equipped with 1 electronic feeder and 1 nipple drinker in a mechanically ventilated room. This grouping approach was used because ADG is the most important determinant of SID Lys requirement (Hauschild et al., 2012) and thus allow the provision of a single diet to all the pigs within a pen. The pens were then randomly assigned within Gr groups to one of the 5 dietary treatments which consisted in CPF with 100% (CPF100) and 4 GPF with 80% (GPF80), 100% (GPF100), 120% (GPF120), and 140% (GPF140) of the ideal Thr:Lys of 0.65 (van Milgen et al., 2008). A 12 h light period was set from 0730 to 1930 h and the room temperature was weekly adjusted from 22 ºC to 18 ºC. Water and feed were provided *ad libitum* during the adaptation and the 21-d experimental period.

2.2 Diets

Dietary treatments (Table 1) were obtained by blending four different pelleted feeds with similar net energy content (A1, B1, A2 and B2). Diets A1 and A2 (high nutrient density) were formulated to meet the SID Lys requirement of the most demanding pig at the beginning of the trial (1.20% SID Lys). Meanwhile, diets B1 and B2 (low nutrient density) were formulated to meet the SID Lys requirement of the least demanding pig at the end of the trial (0.50% SID Lys). Diets A1 and B1 were formulated with 80% and diets A2 and B2 with 140% of the 0.65 Thr to Lys ratio. Minerals and net energy content were provided according to the NRC (2012) for the phase of 25-50 kg BW. All AA, with exception of Lys and Thr, were provided 10% above the following AA to Lys ratios: 30% Met and 60% Met + Cys, 65% Thr, 22% Trp, 70% Val, 52% Ile, 101% Leu, 31% His and 56% Arg (van Milgen et al., 2008; van Milgen and Dourmand, 2015). The feeds total AA

concentration was determined with UHPLC (Masterlab, QC, Canada) and the SID AA concentration (Table 1) was estimated applying the digestibility coefficients obtained with the EvaPig® (version 2.0.3, METEX NØØVISTAGO, INRAE and AFZ, France) on the total AA concentration.

Table 1. Ingredients and chemical composition of the experimental diets A1, A2, B1 and B2 (as-fed basis)[1].

Item, %	A1	A2	B1	B2
Feedstuff				
Corn	58.28	57.80	83.96	83.66
Soybean meal	28.10	28.10	3.50	3.60
Wheat	10.00	10.00	10.00	10.00
Limestone	1.51	1.51	1.28	1.28
Monocalcium phosphate	0.24	0.25	0.29	0.29
Soybean hulls	0.30	0.30	-	-
Salt	0.23	0.23	0.23	0.23
Lysine sulfate	0.59	0.59	0.42	0.42
DL-methionine	0.26	0.26	0.02	0.02
L-tryptophan	0.09	0.09	0.05	0.05
L-threonine	0.02	0.50	-	0.20
L-valine	0.15	0.15	0.03	0.03
Min/vit premix[2]	0.20	0.20	0.20	0.20
Choline chloride	0.02	0.02	0.02	0.02
Phytase[2]	0.01	0.01	0.01	0.01
Calculated chemical composition				
ME, kcal/kg	3,241	3,243	3,213	3,214
NE, kcal/kg	2,410	2,411	2,544	2,544
Dry matter	87.91	87.97	87.22	87.24
Crude protein	19.31	19.62	9.18	9.35
Crude fiber	2.82	2.80	2.44	2.44
Crude fat	2.63	2.61	2.68	2.68
Total Ca	0.74	0.74	0.59	0.59
Total P	0.41	0.41	0.32	0.32
STTD P	0.34	0.34	0.29	0.29
Estimated SID AA[3]				
Lysine	1.13	1.16	0.52	0.50
Methionine	0.45	0.50	0.17	0.14
Methionine + Cysteine	0.70	0.75	0.34	0.32
Threonine	0.68	1.14	0.33	0.46
Tryptophan[4]	0.29	0.29	0.12	0.12
Valine	0.92	0.93	0.43	0.41
Histidine	0.47	0.48	0.23	0.22
Isoleucine	0.71	0.72	0.32	0.30
Leucine	1.47	1.48	0.91	0.88
Phenylalanine	0.85	0.85	0.39	0.38
Arginine	1.17	1.19	0.46	0.43

[1]Feeds A and B were formulated with 1.20% and 0.50% SID Lys, respectively. Feeds 1 and 2 were formulated with 80% and 140% of the ideal Thr:Lys of 0.65 (van Milgen et al., 2008), respectively.

[2]Mineral and vitamin premix supplied per kg of diet (as-fed basis): magnesium, 2000 mg; manganese, 15000 mg; iron, 40000 mg; zinc, 60000; copper, 7500 mg; iodine, 248 mg; selenium, 150 mg; vitamin A, 2000000 UI; vitamin D, 500000 UI; vitamin E, 15000 UI; vitamin K, 1000 mg; vitamin B12, 12500 mcg; riboflavin, 3496 mg; niacin, 12557 mg; folic acid, 243 mg; pyridoxine, 494 mg; thiamine, 495 mg; pantothenic acid, 7343 mg; biotin, 37400 mcg; Phytase, 500 FTU.

[3]Estimated applying the digestibility coefficients obtained with the EvaPig® on the total AA concentrations.

[4]Estimated values used in the feed formulation.

SID = standardized ileal digestible, STTD = standardized total tract digestible.

2.3 Feeding systems

Pigs in the CPF100 treatment were fed with a single diet throughout the trial with 0.97% SID Lys and 0.65 Thr:Lys. This SID Lys concentration was estimated as 120% of the requirement of the average pig (3 d) within the population. This methodology was applied to maximize growth performance of CPF100 pigs (Remus et al., 2019a).

Pigs place in the GPF treatments were fed diets tailored to meet the requirement of the most demanding pig in the pen (2 females and 2 barrows) aiming to avoid nutritionally efficient diets and maximize growth performance of all pigs. The SID Lys requirements of GPF pigs were estimated every two days according to the model proposed by Hauschild et al. (2012). Using real-time data, the model forecast next 2 days ADFI, BW and ADG based on individual pig information collected in real time. Factorial equations are used to estimate the SID Lys requirement for maintenance and growth based on ADFI, BW and ADG estimates. Thus, maintenance Lys requirements were estimated adding the basal endogenous losses (0.313 g Lys/kg DM × ADFI) to the losses related to desquamation in the digestive tract (0.0045 g Lys/kg $BW^{0.75}$ × $BW^{0.75}$), and body protein turn over (0.0239 g Lys/kg $BW^{0.75}$ × $BW^{0.75}$; van Milgen et al., 2008). The SID Lys requirements for growth were calculated assuming that 16% of the ADG was protein (de Lange et al., 2003) and

that 7% of the protein gained was composed of Lys (Mahan and Shields, 1998), and the efficiency of Lys retention from dietary digestible Lys was 72% (Möhn et al., 2000). Therefore, after estimating the highest pig requirement of each pen, the four experimental diets were proportionally blended to achieve the desired SID Lys concentration with the designated Thr:Lys. A mixer with the capacity of 20 kg per batch (Marion Mixers Inc., IA, USA) was used to mix the feeds. The mixing protocol consisted of three cycles of one minute each, with the mixer being emptied between cycles.

2.4 Data collection

2.4.1 Individual requirements estimates

Body weight was individually measured twice a week and individual ADFI was measured daily by the feeding stations. These data were used as inputs in the precision feeding model to estimate the SID Lys requirement of GPF pigs (Hauschild et al., 2012).

2.4.2 Growth performance and body composition

Growth performance was assessed by the evaluation of BW, ADG, ADFI and G:F. All pigs were scanned for the evaluation of total body fat and lean content with dual-energy X-ray absorptiometry equipment (iDXA, GE Lunar Corp., WI, United States of America) at d 1 and 21. Anesthesia was induced with 7% sevoflurane and maintained with 5% isoflurane during the scans. Body lean and fat masses were converted into their protein and lipid chemical equivalents (Kasper et al., 2021). This conversion allowed for the determination of total body protein and lipid, as well as protein deposition (**PD**) and lipid

deposition (**LD**). Additionally, the percentage of PD in ADG was used to determine the PD to ADG ratio (**PD:DG**).

2.4.3 Nitrogen, lysine and threonine efficiencies

Nutrient intake was calculated multiplying the individual ADFI by its respective analysed nutrient content in the blend of feeds provided for each pen. Nitrogen intake and retention were estimated assuming that 6.25% of the dietary CP and PD are N, respectively. Values of N efficiency were expressed as the percentage of the intake that was retained.

Lysine efficiency (**$_k$Lys**) and Thr efficiency (**$_k$Thr**) were calculated as the proportion of AA available retained as protein. The available SID Lys and SID Thr were estimated subtracting the estimated maintenance requirement from the SID AA intake. Maintenance SID Lys requirements were calculated as previously described. The SID Thr maintenance requirements were estimated by adding the basal endogenous losses (0.330 g Thr/kg DM × ADFI), the integument losses (0.033 g Thr/kg $BW^{0.75}$ × $BW^{0.75}$), and the losses resulting from the basal turn over (0.0138 g Thr/kg $BW^{0.75}$ × $BW^{0.75}$) as suggested by van Milgen et al. (2008). Retained values of SID Lys and SID Thr were estimated assuming that 7% and 3.7% of the PD is composed of Lys and Thr, respectively (Mahan and Shields, 1998; de Lange et al., 2001).

2.4.4 Blood sampling and analysis

On d -1, 6 and 20 all pigs were blood sampled with jugular puncture after a minimum of 8 h fasting. Blood collection dates (d -1 and 20) occurred one day before the iDXA to minimize the effect of the blood sampling stress on the effectiveness of the anesthesia protocol. Samples were collected in EDTA, heparin and serum tubes. Plasma tubes were immediately placed on ice after

collection and centrifuged for 15 min at 2000 × g at 4 °C within 20 minutes after sampling. Serum tubes were allowed to clot for 2 h at room temperature and 22 h at 4 °C before centrifugation for 12 min at 1800 × g at 4 °C. Plasma and serum were stored in eppendorfs at -80 °C until analysis. Serum was used to assess the immunoglobulin-g (IgG), C-reactive protein (CRP) and albumin concentrations. The IgG and CRP concentrations were determined with the ELISA kits (Cedarlene, ON, CA and Alpco, NH, USA, respectively). The analysis were performed following the manufacturer guidelines. The albumin serum concentration was analyzed at the Centre de Diagnostic Vétérinaire at the Université de Montréal (Saint-Hyacinthe, Quebec, CA). Plasma samples collected with the EDTA tubes were used to determine the glutathione peroxidase (GPx) activity that was expressed as mU/mL of plasma or mU/mg of plasma proteins. The GPx activity was determined as described by Dalto et al. (2016). Plasma samples collected with heparin tubes were used to determine plasmatic AA concentration which were analyzed as suggested by Remus et al. (2019a). The IgG, CRP, albumin and GPx analysis were performed with the serum collected on days -1 and 20, while the plasmatic AA concentration was determined with the samples collected on days 6 and 20.

2.5 Statistical analysis

The FORECAST procedure of SAS (version 9.4, SAS Institute Inc., North Carolina, USA) was used to estimate individual DG during the adaptation period and to classify pigs as Fast Gr, Medium Gr and Slow Gr. Data were analyzed as a complete randomized block design using the MIXED procedure. Growth rate blocks, treatments and their interactions were used as fixed effects and sex was used as a random effect in the statistical model. The normality of the Student residuals was assessed with the Shapiro-Wilk test using the UNIVARIATE procedure. When the normality assumption was not

met, the data were transformed using the BLOM transformation. Body weight, ADG, body protein mass, body lipids mass, blood parameters and plasmatic AA concentration were analyzed as repeated measures over time. A week effect was used in the analysis of BW and ADG and a sampling day effect was used in the analysis of body protein, body lipids, blood parameters and plasmatic AA concentration. The covariance matrix with the lowest AIC values for the repeated measures analysis was used. When the main effects were statistically significant, polynomial contrasts were used to compare nutritional treatments and Gr blocks. Contrasts were also used to compare the CPF100 and GPF100 treatments. The uncertainty in the estimate of the means of the data was expressed as standard error of means (SEM). The pig was considered the experimental unit. Significant differences were considered when $P \leq 0.05$ and trend towards significance when $0.05 < P \leq 0.10$.

3. Results

3.1 General overview: descriptive analysis

During the trial, one female placed in the GPF80 treatment with Medium Gr had fever and lameness. The pig was medicated and removed from the trial and statistical analysis. All remaining pigs were in good health throughout the trial.

The overall 21-day average estimated individual SID Lys requirement of Fast, Medium and Slow Gr pigs within GPF treatments were 0.85%, 0.89% and 0.90%, respectively. This resulted in a SID Lys intake of 24, 24 and 23 g/d, respectively. Feeding pigs based on the most demanding pig of the pen resulted in GPF pigs receiving, on average, diets with 11% more SID Lys concentration than their individual requirements. Meanwhile, CPF100 pigs were fed with 17% more SID Lys than their individual

requirements when provided a single-phase diet formulated to be 20% above the average pig requirement.

The initial coefficient of variation in the pig's BW on d 1 within the GPF100, GPF120, and GPF140 treatments were 7%, 8%, and 8%, respectively, and decreased over time being 4%, 5% and 6%, respectively. In contrast, the coefficient of variation for CPF100 and GPF80 were 9% and 8% on d 1. These variations remained relatively stable over time, ending on d 21 at 10% and 8%, respectively.

3.2 Growth performance and body composition

Due to the study design, the linear, quadratic, and cubic effects of Thr level were only evaluated within the GPF treatments. Consequently, any reported main effect of Thr level should be understood as applying specifically to the GPF treatments. The reference control treatments CPF100 and GPF100 were compared to evaluate whether there was an interaction between these treatments and Gr.

No initial difference ($P > 0.10$) in BW, body protein and body lipid masses was observed among dietary treatments or Gr (Table 2). A linear Thr × linear-quadratic Gr interaction was observed ($P \leq 0.05$) for the initial ADG.

No difference between CPF100 and GPF100 was found in the overall growth performance or final body conditions. In the overall growth performance, linear Thr × linear Gr interactions were observed for both ADG and ADFI ($P \leq 0.05$). Within GPF80 and GPF100, ADG increased linearly with faster Gr. In contrast, within the GPF120 and GPF140 treatments, ADG increased linearly with slower Gr. The ADG of Fast and Medium Gr pigs was similar across treatments, while the ADG of Slow Gr increased linearly with

the higher Thr levels, resulting in a similar ADG to that of Fast and Medium pigs. For ADFI, the GPF100 and GPF80 treatments showed a linear increase with faster Gr, while in the GPF120 and GPF140 treatments, ADFI increased linearly with slower Gr. The G:F presented a cubic increase in response to the Thr level ($P \leq 0.05$) independent of the Gr.

The PD was not affected by Thr level or Gr. However, a tendency ($P \leq 0.10$) for a linear Thr × linear Gr interaction was observed for LD. The LD of GPF80 and GPF100 pigs linearly decreased with the slower Gr. No differences in the LD of GPF120 and GPF140 within Gr was observed. Overall, a linear decrease ($P \leq 0.05$) of LD was observed with faster Gr. Similarly, lean gain, presented as the PD:DG, increased in a cubic manner with increasing Thr levels and linearly decreased with faster Gr. Thus, Slow Gr seemed to be leaner than Fast Gr in this trial. This was highlighted by the final body lipid mass which presented a linear decrease ($P \leq 0.05$) with slower Gr. Similarly, body protein mass tended ($P \leq 0.10$) to present a linear increase with increasing Thr levels and a linear decrease with slower Gr. The body protein content of GPF80 pigs decreased linearly with decreasing Gr, while it linearly increased as the Gr decreased within GPF140. The body protein content of GPF100 and GPF120 remained equal between Gr. Finally, final BW decreased linearly ($P \leq 0.05$) with slower Gr, but no difference was observed among Thr levels.

Table 2. Performance and body composition of Fast (F), Medium (M), and Slow (S) growth rate pigs fed with either conventional phase-feeding or graded Thr-to-Lys ratios within group precision feeding[1].

	CPF100			GPF80			GPF100			GPF120			GPF140				*P*-value[2]		
Item	F	M	S	F	M	S	F	M	S	F	M	S	F	M	S	SEM	Thr	Gr	Thr × Gr
Initial conditions																			
BW, kg	48.08	45.20	41.20	48.05	44.63	43.53	42.30	44.23	43.25	44.50	44.63	43.33	45.88	43.78	47.20	0.468	0.46	0.18	0.24
Body protein, kg	7.67	7.24	6.55	7.60	7.08	6.86	6.68	7.04	6.85	6.99	6.97	6.88	7.36	6.92	7.56	0.076	0.32	0.19	0.17
Body lipids, kg	6.44	5.66	5.09	6.80	5.98	5.93	5.61	5.70	5.69	6.32	6.55	5.63	5.72	5.88	6.06	0.118	0.47	0.23	0.64
ADG, g/d	1063	912	831	1068	894	882	1017	938	869	1035	951	833	1003	968	840	11.32	0.77	<0.01	**<0.01**[¥§]
Final conditions																			
BW, kg	75.25	69.03	62.65	73.80	70.23	64.43	69.30	68.23	65.65	69.08	68.08	67.80	70.63	70.40	73.38	0.647	0.40	**0.02**[†]	0.12
Body protein, kg	11.59	10.92	9.93	11.26	10.70	10.07	10.68	10.76	10.28	10.44	10.31	10.53	11.01	10.62	11.47	0.095	0.25	**0.07**[†]	**0.07**[¥]
Body lipids, kg	14.73	11.45	9.98	14.97	14.14	11.17	13.23	11.57	11.29	14.38	13.91	12.37	12.74	14.77	13.28	0.306	0.20	**<0.01**[†]	0.37
Overall performance																			
ADG, g/d	1297	1223	1048	1212	1236	1021	1303	1167	1063	1169	1130	1198	1174	1283	1315	15.40	0.12	<0.01	**<0.01**[¥]
ADFI, g/d	2968	2527	2240	2929	2804	2313	2752	2444	2356	2876	2684	2669	2683	2986	2777	41.60	0.03	<0.01	**0.02**[¥‡]
G:F, g/g	0.435	0.450	0.458	0.418	0.433	0.433	0.468	0.473	0.453	0.408	0.415	0.440	0.440	0.428	0.453	0.004	**<0.01**[¶]	0.30	0.71
PD, g/d	187	176	161	174	173	153	191	177	163	164	159	174	174	176	186	2.287	0.18	0.13	0.12
LD, g/d	395	276	232	389	385	250	363	279	267	384	351	321	334	423	344	10.78	**0.06**	**<0.01**[†]	**0.07**[¥‡]
PD:DG, %	14.4	15.5	15.8	14.3	14.2	15.4	14.8	15.6	15.4	14.0	14.2	14.9	14.8	13.9	15.0	0.123	**0.03**[¶]	**0.01**[†]	0.46

CPF = conventional group-phase feeding; GPF = group precision feeding; Thr = Threonine level; Gr = growth rate; SEM = standard error of means; PD = protein deposition; LD = lipid deposition; PD:DG = protein deposition to daily gain ratio.

[1]Treatments consisted of CPF with 100% (CPF100) and GPF with 80% (GPF80), 100% (GPF100), 120% (GPF120), and 140% (GPF140) of the ideal Thr-to-Lys ratio of 0.65 (van Milgen et al., 2008).

[2]Statistical models of final conditions variables also included time (T) and interactions with Thr and Gr per variable. BW: T ≤ 0.05, Thr×T = 0.74, Gr×T = 0.62, Thr×Gr×T = 0.31; Body protein: T ≤ 0.05, Thr×T = 0.18, Gr×T = 0.14, Thr×Gr×T = 0.12; Body lipids: T ≤ 0.05, Thr×T = 0.07, Gr×T ≤ 0.05, Thr×Gr×T = 0.08.

[†]Linear main effect; [¶]Cubic main effect ($P \leq 0.05$).

[¥]Linear Thr level × linear Gr; [§]Linear Thr level × quadratic Gr; [‡]Quadratic Thr level × quadratic Gr ($P \leq 0.05$).

Values are least square means.

A linear Gr × linear time interaction was observed ($P \leq 0.05$) for the ADG. The ADG of Slow and Medium Gr pigs linearly decreased over the experimental weeks, whereas it linearly increased for pigs in the Fast Gr. The linear Gr × linear time interaction was also observed for the body lipid mass in which the linear increase in body lipid content of Fast and Medium pigs was more pronounced than in Slow pigs.

3.3 Nutrient intake and efficiency

The nutrients intake and efficiency are presented in Table 3. A significant linear Thr × linear Gr interaction was observed ($P \leq 0.05$) for CP intake and Lys intake. A linear decrease was observed for fast and medium Gr with the increased Thr levels. In contrast, Thr levels linearly increased the CP intake of Slow pigs. The CP intake of GPF80 and GPF100 linearly increased with faster Gr, whereas it increased in Slow Gr pigs within GPF120 and GPF140. Increasing the Thr level linearly decreased the Lys intake of Fast and Medium growers whereas it increased the Lys intake of Slow Gr pigs. Within GPF80 and GPF100, Lys intake linearly increased with Gr. In contrast, the Lys intake of GPF120 and GPF140 linearly increased with the slow growth. A tendency of linear Thr × linear Gr interaction was observed ($P \leq 0.10$) for Thr intake. The Thr levels linearly increased the Thr intake of Fast, Medium and Slow Gr pigs. However, Thr intake only varied within GPF80, in which it increased with faster growth. No differences in the Thr intake were observed between Fast, Medium and Slow Gr pigs within GPF100, GPF120 and GPF140.

Threonine efficiency decreased ($P \leq 0.05$) linearly with the increasing Thr levels. A tendency ($P \leq 0.10$) of linear decrease of N excretion was observed for Gr where it decreased with the slower growth. The contrasts between CPF100 and GPF100 indicated

that GPF100 pigs excreted 15% less N ($P \leq 0.05$). Furthermore, GPF100 pigs tended to reduce CP intake by 7% ($P \leq 0.10$), Lys intake by 8% ($P \leq 0.10$) and Thr intake by 8% ($P \leq 0.10$). These differences in nutrient intake and excretion resulted in a 10% increase ($P \leq 0.05$) in N efficiency and Lys efficiency of GPF100 compared to CPF100.

Table 3. Nutrient efficiency of Fast (F), Medium (M), and Slow (S) growth rate pigs fed with either conventional phase-feeding or graded Thr-to-Lys ratios within group precision feeding[1].

	CPF100			GPF80			GPF100			GPF120			GPF140				*P*-value[2]		
Item	F	M	S	F	M	S	F	M	S	F	M	S	F	M	S	SEM	Thr	Gr	Thr × Gr
CP intake, g/d$^{\beta}$	478	406	365	426	418	354	393	389	381	391	400	399	395	405	403	5.730	0.49	0.02	**0.05**$^{¥}$
N excretion, g/d$^{\alpha}$	18.2	14.4	12.8	15.7	15.4	12.6	12.7	13.2	13.6	14.2	15.1	14.1	13.8	14.3	13.6	0.330	0.33	**0.10**†	0.21
N efficiency, %$^{\beta}$	39.2	43.4	44.4	41.2	41.4	43.5	48.5	46.4	43.2	42.0	39.9	44.0	44.0	43.6	46.5	0.662	0.18	0.48	0.54
Lys intake, g/d$^{\beta}$	28.8	24.5	22.0	25.5	25.1	21.3	23.3	23.4	23.0	22.8	23.7	23.6	23.2	23.5	23.6	0.347	0.28	0.03	**0.04**$^{¥}$
$_{k}$Lys, %$^{2\beta}$	47.9	53.2	54.5	51.2	51.1	53.7	60.9	57.0	53.0	53.7	50.0	55.1	55.7	56.1	59.1	0.851	0.12	0.53	0.40
Thr intake, g/d$^{\beta}$	18.6	15.8	14.2	13.2	13.0	11.0	15.1	15.1	14.9	17.8	18.5	18.4	21.1	21.3	21.4	0.445	**<0.01**†	**0.06**†	**0.06**$^{\downarrow}$
$_{k}$Thr, %^{3}	39.8	44.2	45.2	54.1	53.9	56.7	50.6	47.3	44.0	36.6	34.0	37.5	32.2	32.5	34.2	1.203	**<0.01**†	0.51	0.60

CPF = conventional group-phase feeding; GPF = group precision feeding; Thr = Threonine level; Gr = growth rate; SEM = standard error of means.

[1]Treatments consisted of CPF with 100% (CPF100) and GPF with 80% (GPF80), 100% (GPF100), 120% (GPF120), and 140% (GPF140) of the ideal Thr-to-Lys ratio of 0.65 (van Milgen et al., 2008).

[2]SID Lys utilization efficiency ($_{k}$Lys) = (PD × 0.07) / (SID Lys intake – ((0.313 g Lys/kg DM × ADFI) + (0.0045 g Lys/kg $BW^{0.75}$ × $BW^{0.75}$) + (0.0239 g Lys/kg $BW^{0.75}$ × $BW^{0.75}$))) × 100, where PD is protein deposition, DM is dry-matter, DFI is daily feed intake and BW is body weight.

[3]SID Thr utilization efficiency ($_{k}$Thr) = (PD × 0.037) / (SID Thr intake – ((0.330 g Thr/kg DM × ADFI) + (0.033 g Thr/kg $BW^{0.75}$ × $BW^{0.75}$) + (0.0138 g Thr/kg $BW^{0.75}$ × $BW^{0.75}$))) × 100, where PD is protein deposition, DM is dry-matter, ADFI is average daily feed intake and BW is body weight.

†Linear main effect ($P \leq 0.05$).

$^{¥}$Linear Thr level × linear Gr ($P \leq 0.05$).

$^{\downarrow}$Tendency for linear Thr level × linear Gr ($0.05 < P \leq 0.10$).

$^{\alpha}$Contrast between CPF100 and GPF100 ($P \leq 0.05$).

$^{\beta}$Tendency for contrast between CPF100 and GPF100 ($0.05 < P \leq 0.10$).

Values are least square means.

3.4 Blood parameters and plasmatic AA concentration

The blood parameters concentration are shown in Table 4 and the plasmatic AA concentration on Table 5. There was a tendency for a cubic effect ($P \leq 0.10$) of Gr on the IgG concentration on d -1. Similarly, we observed a tendency for a cubic effect ($P \leq 0.10$) of the Thr levels on the CRP concentration on d -1. In contrast, we did not observe a Thr level or Gr effect ($P > 0.10$) on the other blood parameters on the statistical analysis performed. No differences ($P > 0.10$) were found in the contrasts between GPF100 and CPF100.

Significant linear Thr × linear-quadratic Gr interaction ($P \leq 0.05$) was observed on d 20 for Thr plasmatic concentration. A linear Gr × linear time interaction ($P \leq 0.05$) was observed for the Thr plasma concentration. The plasmatic Thr concentration of Medium and Slow Gr pigs linearly decreased from d 6 to 20, while it increased in Fast pigs. We observed a linear Thr x linear time interaction ($P \leq 0.05$) for the Lys plasma concentration. The plasma Lys concentration of GPF140 decreased from d 6 to 20. In contrast, the Lys plasma concentration increased in GPF80, GPF100 and GPF120 from d 6 to 20.

The Thr levels linearly increased the Thr plasma concentration of fast, medium and slow-growing pigs. The faster growth rate linearly decreased the Thr plasma concentration of GPF80 and GPF100 pigs, whereas it increased the Thr plasma concentration of GPF120 and GPF140 treatments. The increased Thr levels tended ($P \leq 0.10$) to decrease linearly the Lys plasma concentration. No differences ($P > 0.10$) in the plasma Thr or Lys concentration were observed between GPF100 and CPF100.

Table 4. Blood parameters of Fast (F), Medium (M), and Slow (S) growth rate pigs fed with either conventional phase-feeding or graded Thr-to-Lys ratios within group precision feeding[1].

	CPF100			GPF80			GPF100			GPF120			GPF140				*P*-value[2]		
Item	F	M	S	F	M	S	F	M	S	F	M	S	F	M	S	SEM	Thr	Gr	Thr × Gr
Day -1																			
IgG, mg/mL	6.04	7.34	7.43	5.64	5.77	7.87	7.19	5.04	6.29	5.87	7.03	8.33	8.59	5.56	7.01	0.243	0.64	**0.09**¶	0.12
GPx, mU/mL[3]	1950	1738	1825	1762	1898	1932	1963	2078	2025	1925	2018	1882	2004	1902	1796	25.60	0.18	0.84	0.49
GPx, mU/mg[3]	29.5	27.9	26.9	29.8	27.9	31.5	30.2	27.8	31.2	32.0	33.2	31.6	26.6	29.7	28.5	0.539	0.12	0.91	0.80
CRP, µg/mL	318	384	414	187	294	175	288	430	325	271	200	213	435	264	212	21.52	**0.08**¶	0.30	0.35
Albumin, g/L	36.9	35.3	36.4	34.3	35.9	35.8	35.8	34.9	34.6	36.3	34.3	34.1	36.7	35.3	35.6	0.409	0.89	0.72	0.98
Day 20																			
IgG, mg/mL	7.77	8.94	8.16	5.91	7.47	8.88	7.78	5.87	6.91	6.08	8.40	8.47	9.62	6.68	8.87	0.283	0.49	0.20	0.14
GPx, mU/mL[3]	2062	1984	1917	2004	2009	1995	2122	2118	2181	2019	1990	1970	2136	1948	2001	26.12	0.25	0.78	0.88
GPx, mU/mg[3]	29.5	32.9	27.1	32.3	30.3	30.2	29.6	28.5	28.2	33.1	34.3	29.0	28.7	31.5	30.0	0.594	0.24	0.71	0.80
CRP, µg/mL	199	244	161	131	302	111	190	258	175	271	263	194	195	206	201	15.64	0.28	0.12	0.46
Albumin, g/L	40.7	36.2	37.3	37.1	37.6	35.0	36.2	36.3	36.3	38.3	35.9	35.4	36.3	38.7	39.7	0.434	0.35	0.42	0.87

CPF = conventional group-phase feeding; GPF = group precision feeding; Thr = Threonine level; Gr = growth rate; SEM = standard error of means; IgG = immunoglobulin G; GPx = glutathione peroxidase; CRP = C-reactive protein.

[1]Treatments consisted of CPF with 100% (CPF100) and GPF with 80% (GPF80), 100% (GPF100), 120% (GPF120), and 140% (GPF140) of the ideal Thr-to-Lys ratio of 0.65 (van Milgen et al., 2008).

[2]Statistical models of the Day 20 variables also included time (T) and interactions with Thr and Gr per variable. IgG: $T \leq 0.05$, Thr×T = 0.37, Gr×T = 0.32, Thr×Gr×T = 0.64; GPx (mU/mL): $T \leq 0.05$, Thr×T = 0.54, Gr×T = 0.42, Thr×Gr×T = 0.37; GPx (mU/mg): T = 0.20, Thr×T = 0.41, Gr×T = 0.07, Thr×Gr×T = 0.91; CRP: $T \leq 0.05$, Thr×T = 0.45, Gr×T = 0.70, Thr×Gr×T = 0.62; Albumin: $T \leq 0.05$, Thr×T = 0.78, Gr×T = 0.80, Thr×Gr×T = 0.20.

[3]GPx was expressed as mU per mL of plasma and mU per mg of plasmatic proteins.

¶Cubic main effect ($P \leq 0.05$).

Values are least square means.

Table 5. Plasmatic free AA concentration of Fast (F), Medium (M), and Slow (S) growth rate pigs fed with either conventional phase-feeding or graded Thr-to-Lys ratios within group precision feeding[1].

	CPF100			GPF80			GPF100			GPF120			GPF140				*P*-value[2]		
Item, µmol/L	F	M	S	F	M	S	F	M	S	F	M	S	F	M	S	SEM	Thr	Gr	Thr × Gr
Day 6																			
Thr	67.3	68.3	60.6	51.4	52.8	40.6	59.1	60.5	69.9	76.8	75.9	75.5	108.5	78.3	108.0	2.743	**<0.01**	0.36	**0.03**[§]
Lys	38.3	34.2	43.9	47.3	42.0	35.9	34.9	47.9	43.0	43.2	45.0	36.4	42.1	37.3	35.4	1.363	0.87	0.74	0.39
Day 20																			
Thr	69.2	66.4	61.0	56.1	65.2	34.6	65.6	53.2	65.8	81.3	69.2	74.5	115.4	75.1	85.8	2.739	<0.01	0.05	**0.01**[¥§]
Lys	43.3	44.8	51.4	53.7	58.0	46.7	42.5	44.7	42.4	43.2	46.2	47.8	34.4	35.0	30.0	1.520	**0.07**[†]	0.77	0.61

CPF = conventional group-phase feeding; GPF = group precision feeding; Thr = Threonine level; Gr = growth rate; SEM = standard error of means; IgG = immunoglobulin G; GPx = glutathione peroxidase; CRP = C-reactive protein.

[1]Treatments consisted of CPF with 100% (CPF100) and GPF with 80% (GPF80), 100% (GPF100), 120% (GPF120), and 140% (GPF140) of the ideal Thr-to-Lys ratio of 0.65 (van Milgen et al., 2008).

[2]Statistical models of the Day 20 variables also included time (T) and interactions with Thr and Gr per variable. Thr: T = 0.53, Thr×T = 0.43, Gr×T ≤ 0.05, Thr×Gr×T = 0.20; Lys: T ≤ 0.05, Thr×T ≤ 0.05, Gr×T = 0.76, Thr×Gr×T = 0.71.

[†]Linear main effect ($P \leq 0.05$).

[¥]Linear Thr level × linear Gr; [§]Linear Thr level × quadratic Gr ($P \leq 0.05$).

Values are least square means.

4. Discussion

4.1 Growth rate is affected by Threonine ratios

The precision feeding model uses the growth pattern of each pig to forecast BW, ADG and ADFI. These parameters are then applied in factorial equations to estimate the individual SID Lys requirement that supports the predicted growth (Hauschild et al., 2012). After determining the SID Lys requirement, other essential AA are assumed to be required at fixed proportions relative to Lys, following the ideal protein concept (van Milgen and Dourmand, 2015). As ADFI tends to increase more than proportionally to ADG, the concentration of SID Lys and other essential AA gradually diminish, potentially limiting growth. This suggests that higher AA ratios might compensate for this deficiency, possibly explaining the improved performance observed in pigs fed with GPF140. However, the performance of GPF100 and CPF100 did not differ significantly across any growth variables. Furthermore, the absence of interactions between treatments and Gr in the contrast of GPF100 and CPF100 demonstrate that the growth of fast, medium and slow Gr pigs are equal within these treatments. These findings are aligned with previous research (Andretta et al., 2014, 2016; Santos et al., 2018), and confirms that, similar performance is achieved when feeding pigs with GPF or CPF despite the reduction in dietary AA concentrations over time.

The ideal protein concept used in feed formulation is based on the principle that optimal growth is achieved when all AA are provided in the correct proportions (van Milgen and Dourmand, 2015). If one or more essential AA are not supplied in adequate amounts, the deficient AA becomes the limiting factor for growth. Therefore, increasing the ratio of just one AA above the optimal ratio established for conventional group-phase

feeding may not further enhance growth performance. Additionally, since the precision feeding model accounts for individual ADG, Thr ratios might not result in different performances across Gr categories. Interestingly, in this experiment, not only did increasing Thr:Lys ratios enhance performance, but the degree to which performance was influenced also varied according to the Gr category. Specifically, the interaction between feeding treatments and Gr indicates that the ADG of Slow Gr pigs was influenced by the increased Thr ratios but it did not influence the ADG of Fast and Medium Gr.

The slow growth observed in certain pigs is influenced by multiple factors, including genetics, birth weight, environment, health, management, and nutrition (Magowan et al., 2007; Douglas et al., 2014a,b; López-Vérge et al., 2018; Aymerich et al., 2020). Slow-growing pigs tend to exhibit lower digestibility coefficients (Jones and Patience, 2014), reduced plasma AA concentrations, and lower serum levels of anabolic hormones as IGF-1 (He et al., 2016). Research indicates that feeding slow-growing pigs diets with higher nutrient concentration may mitigate some of the growth discrepancies (Aymerich et al., 2020; Douglas et al., 2014a). For example, increasing Thr ratios was shown to linearly elevate plasma Thr concentrations, a molecule known to upregulate plasma IGF-1 (Katsumata et al., 2004), potentially contributing to an increase in ADG. Furthermore, the cubic effect observed in the PD:DG variable indicates a relative increase in protein deposition at GPF140, possibly driven by the higher Thr ratios. This is further supported by a linear decrease in plasma Lys concentrations, as Thr ratios increased, suggesting a shift towards enhanced protein synthesis (Bertolo et al., 2005). The results suggest that optimizing threonine ratios can specifically benefit slow-growing pigs.

However, it is important to consider that the effects observed herein may not be solely due to the Thr ratios, but also to how pigs respond within precision feeding.

To the best of our knowledge, this is the fourth experiment evaluating the effect of AA ratios on pigs fed with precision feeding (Remus et al., 2019b,c; Remus et al., 2020) and the first to assess whether these responses were influenced by the Gr category. Consistent with our findings, previous studies reported improved performance in precision fed pigs with AA ratios higher than those estimated for group-phase feeding (Remus et al., 2019b,c). Moreover, it has been demonstrated that the muscle AA composition can be significantly altered by different AA ratios, as observed by Remus et al. (2019c), suggesting that pigs may adjust their physiological functions in respond to dietary composition. These results highlight the complexity of optimizing AA ratios in precision feeding and the potential for physiological adaptations to dietary changes. However, these findings also raise important questions about the conventional approach to formulating ideal protein diets in swine nutrition. Traditional formulations often rely on fixed AA ratios based on group-phase feeding standards, which may not be fully applicable to precision feeding systems where individual animal responses vary significantly.

Altogether, our results demonstrate that the response of pigs to different Thr:Lys ratios is influenced by the Gr category. The results of this experiment suggests that pigs require distinct AA ratios to maximize growth. The use of a single ratio for precision feeding proved suboptimal, even with the current model accounting for variations over time and among individuals. Consequently, traditional approaches, such as the ideal protein concept, may not be the most effective for optimizing pig performance in precision feeding. Future research should focus on developing new methods to estimate AA requirements

within precision feeding systems. It is important to mention, that the multifactorial causes of slow growth suggests that a single feeding strategy may not be sufficient to address all growth-related issues. The experiment presented herein was conducted in a well-controlled environment in which minimal or no immune, environmental or nutritional stress were observed. Furthermore, the average of ADG of Slow Gr pigs throughout the trial was 1099 g/d. This value is lower than the ADG of the other categories but still represent a good growth parameter. Therefore, the good response of Slow Gr to the increased Thr ratios might also have been possible due to the good experimental conditions used in the trial.

4.2 Effect of Threonine ratios on blood parameters

The concentration of serum proteins serves as an indicator of the nutritional status in pigs. For example, an increase in dietary Thr has been associated with elevated IgG levels, reflecting enhanced immune function (Defa et al., 1998). Similarly, C-reactive protein (CRP), a key acute-phase protein composed of serine, glycine, and threonine (Oliveira et al., 1979), is influenced by dietary Thr intake, which in turn affects its serum concentration (Remus et al., 2019c). Additionally, albumin, a major plasma protein, is dependent on adequate dietary Thr for its synthesis. These relationships highlight the critical role of threonine in modulating protein synthesis and overall health status. Consequently, we anticipated that increases in threonine intake would elevate the synthesis rates of IgG, CRP, and albumin. However, we did not observe a consistent increase in IgG, CRP or albumin concentrations with varying Thr to Lys ratios. The absence of significant effects on IgG and CRP may be attributed to the pigs being raised under optimal conditions without immunological challenges, which likely reduced the stimuli for IgG and CRP

production. Albumin is primarily synthesized in the liver, and its plasma concentration can be influenced by multiple factors, including the availability of other essential amino acids, overall nutritional status, and the presence of inflammatory conditions (Kumar et al., 2014). Therefore, even with increased Thr intake, albumin synthesis may not significantly change if these factors do not promote higher protein production. Thus, the blood variables measured in this experiment do not provide conclusive insights into the specific physiological mechanisms underlying the increased ADG observed in Slow Gr pigs.

4.3 Contrasts between group-phase and precision feeding

Previous studies demonstrated that the use of precision feeding can reduce nutrient excretion and increase nutrient efficiency while maintaining performance (Andretta et al., 2014). However, the differences between GPF100 and CPF100 pigs observed herein are lower than the 25% reduction in SID Lys intake and 30% reduction in N excretion observed by Andretta et al. (2014). In this experiment, GPF pigs were fed based on the requirement of the most demanding pig in the pen. Therefore, it was expected that GPF pigs would ingested slightly higher amounts of SID Lys than their individual requirement. This artefact might have contributed to the low difference observed in the nutrient balance between GPF100 and CPF100 treatments. Nonetheless, the contrasts between GPF100 and CPF100 elicited a similar performance between these treatments with GPF100 pigs ingesting 6.8% less CP, 7.4% less SID Lys, reduced N excretion by 13.9% and increased $_{k}$Lys by 9.0%.

The plasmatic Lys and Thr concentrations were similar between GPF100 and CPF100, despite GPF100 pigs ingesting less SID Lys. Since, we did not evaluate protein synthesis and degradation, we cannot determine whether GPF100 and CPF100 have the

same protein synthesis rate. However, the absence of differences in the plasmatic concentration aligned with the growth performance and nutrient efficiency data, supports the idea that overfeeding pigs with group-phase feeding results in nutrient excretion without improving protein deposition.

Since the conversion of AA into urea requires energy (Musharaf and Latshaw, 1999), we expected that the excess of AA provided to CPF100 pigs would produce more reactive oxygen species, potentially reducing the GPx concentration (Addabbo et al., 2009). However, feeding pigs with GPF100 or CPF100 did not alter serum GPx concentration. These data suggests that the differences in the dietary AA concentration were not enough to induce changes in the pigs' antioxidant defences. Another possible explanation for the GPx results could be the similar energy intake of GPF100 and CPF100. High energy density diets increase the production of reactive oxygen species (Addabbo et al., 2009), leading to greater oxidation (Adebowale et al., 2019). However, in this experiment, the net energy did not differ between the four experimental diets. Maintaining similar energy content across diets is a common practice in precision feeding to prevent large fluctuations in dietary energy, especially because the SID Lys concentration is adjusted daily. Thus, even receiving different amounts of AA, pigs were fed the same net energy concentration. The results of this experiment corroborates the findings of other authors, indicating that overfeeding pigs with group-phase feeding increases nutrient excretion without improving the performance (Andretta et al., 2014) or altering Lys and Thr plasmatic concentration (Remus et al., 2019a).

5. Conclusions

In conclusion, the response of pigs fed different Thr-to-Lys ratios within group-precision feeding is influenced by their growth rate. Increasing the Thr ratios from 80% to 140% of the ideal 0.65 improved the ADG in slow-growing pigs but did not affect those in the fast and medium growth rate categories. Our data do not allow to conclude which physiological mechanism was responsible for the improved performance of slow-growing. However, the results of this experiment point to the conclusion that traditional concepts, such as ideal protein, may not be the most suitable for formulating precision feeding diets. Therefore, there is a need to explore and develop new methods for estimating individual AA requirements that are more responsive to the dynamic nature of precision feeding. Future research should focus on refining these estimation techniques, possibly integrating real-time growth data and advanced modeling approaches, to better tailor dietary formulations to the specific needs of each animal. This could lead to more precise and effective feeding strategies, ultimately enhancing overall performance and productivity.

Conflict of Interest Statement

The authors declares no real or perceived conflicts of interest.

Literature Cited

Addabbo, F., Montagnani, M., & Goligorsky, M. S. (2009). Mitochondria and reactive oxygen species. *Hypertension*, *53*(6), 885-892.

Adebowale, T., Shunshun, J., & Yao, K. (2019). The effect of dietary high energy density and carbohydrate energy ratio on digestive enzymes activity, nutrient digestibility, amino acid utilization and intestinal morphology of weaned piglets. *Journal of Animal Physiology and Animal Nutrition*, *103*(5), 1492-1502.

Andretta, I., Pomar, C., Rivest, J., Pomar, J., Lovatto, P. A., & Radünz Neto, J. (2014). The impact of feeding growing–finishing pigs with daily tailored diets using precision feeding techniques on animal performance, nutrient utilization, and body and carcass composition. *Journal of Animal Science,* 92(9), 3925-3936.

Andretta, I., C. Pomar, J. Rivest, J. Pomar, and J. Radünz. 2016. Precision feeding can significantly reduce lysine intake and nitrogen excretion without compromising the performance of growing pigs. *Animal,* 10(7):1137-1147.

Aymerich, P., Soldevila, C., Bonet, J., Gasa, J., Coma, J., & Solà-Oriol, D. (2020). Increasing dietary lysine impacts differently growth performance of growing pigs sorted by body weight. *Animals*, *10*(6), 1032.

Barea, R., Pujols, J., & Figueroa, J. (2009). Variability in growth responses to amino acid ratios in pig production: A review. *Livestock Science*, 123(2-3), 266-273

Bertolo, R. F., Moehn, S., Pencharz, P. B., & Ball, R. O. (2005). Estimate of the variability of the lysine requirement of growing pigs using the indicator amino acid oxidation technique. *Journal of Animal Science*, *83*(11), 2535-2542.

Cadéro, R., Pallarés, J., & Fernández, A. (2020). Sanitary challenges and their effects on growth performance in pig production**.** *Animal Production Science*, 60(6), 724-734.

Cloutier, L., Pomar, C., Montminy, M. L., Bernier, J. F., & Pomar, J. (2015). Evaluation of a method estimating real-time individual lysine requirements in two lines of growing–finishing pigs. *Animal*, 9(4), 561-568.

Dalto, D. B., Mores, N., & Lobo, R. (2016). Assessment of GPx activity in biological samples: Methodological approaches. *Analytical Biochemistry*, 511, 42-48.

Davoudkhani, H., Hossain, M., & Koenig, K. (2020). Strategies for improving herd homogeneity and their impact on economic and environmental sustainability in pig production. *Journal of Cleaner Production*, 276, 124191.

de Lange, C. F. M., Gillis, A. M., & Simpson, G. J. (2001). Influence of threonine intake on whole-body protein deposition and threonine utilization in growing pigs fed purified diets. *Journal of Animal Science*, 79, 3087-3095.

de Lange, C. F. M., Morel, P. C. H., & Birkett, S. H. (2003). Modeling chemical and physical body composition of the growing pig. *Journal of Animal Science*, *81*(14_suppl_2), E159-E165.

Defa, L., Changting, X., Shiyan, Q., Jinhui, Z., Johnson, E. W., & Thacker, P. A. (1999). Effects of dietary threonine on performance, plasma parameters and immune function of growing pigs. *Animal Feed Science and Technology*, *78*(3-4), 179-188.

Douglas, S. L., Wellock, I., Edwards, S. A., & Kyriazakis, I. (2014a). High specification starter diets improve the performance of low birth weight pigs to 10 weeks of age. *Journal of Animal Science*, *92*(10), 4741-4750.

Douglas, J. A., McGlone, J. J., & Morrow, R. (2014b). Management practices to enhance growth and reduce variability in pigs. *Livestock Science*, 162, 142-150.

Hauschild, L., Pomar, C., & Lovatto, P. A. (2010). Systematic comparison of the empirical and factorial methods used to estimate the nutrient requirements of growing pigs. *Animal*, 4(5), 714-723.

Hauschild, L., Lovatto, P. A., Pomar, J., & Pomar, C. (2012). Development of sustainable precision farming systems for swine: estimating real-time individual amino acid requirements in growing-finishing pigs. *Journal of Animal Science*, 90(7), 2255-2263.

He, Y., Deen, J., Shurson, G. C., Wang, L., Chen, C., Keisler, D. H., & Li, Y. Z. (2016). Identifying factors contributing to slow growth in pigs. *Journal of Animal Science*, *94*(5), 2103-2116.

Jones, C. K., & Patience, J. F. (2014). Digestibility coefficients and their relationship with growth rate in pigs. *Journal of Animal Science*, 92(6), 2472-2482.

Kasper, C., Schlegel, P., Ruiz-Ascacibar, I., Stoll, P., & Bee, G. (2021). Accuracy of predicting chemical body composition of growing pigs using dual-energy X-ray absorptiometry. *Animal*, 15(8), 100307.

Katsumata, M., Tanaka, T., & Suzuki, T. (2004). Effects of threonine intake on plasma threonine concentrations and IGF-1 levels in growing pigs. *Journal of Animal Science*, 82(6), 1757-1763.

Kumar, P., S. B. Kaur, and M. B. Munro. (2014). "Albumin: A review of its physiology and clinical significance." *Journal of Clinical Biochemistry and Nutrition*, 54(1), 1-7.

Llorens, J., López, C., & Fernández, A. (2024). Environmental impact of feeding strategies in pig production: A comparison of precision feeding and conventional systems**.** *Journal of Cleaner Production*, 410, 137042.

López-Vérge, J., Gil, J. V., & Gómez, J. (2018). Effects of early life conditions on growth performance in pigs**.** *Journal of Animal Science*, 96(10), 4061-4070.

Magowan, E., McDonald, T., & O'Neill, D. (2007). Influence of genetics and management on growth rates in pigs. *Journal of Animal Science*, 85(3), 455-463.

Mahan, D. C., & Shields Jr, R. G. (1998). Essential and nonessential amino acid composition of pigs from birth to 145 kilograms of body weight, and comparison to other studies. *Journal of Animal Science*, *76*(2), 513-521.

Möhn, S., Gillis, A. M., Moughan, P. J., & De Lange, C. F. M. (2000). Influence of dietary lysine and energy intakes on body protein deposition and lysine utilization in the growing pig. *Journal of Animal Science*, *78*(6), 1510-1519.

Musharaf, N. A., & Latshaw, J. D. (1999). Heat increment as affected by protein and amino acid nutrition. *World's Poultry Science Journal*, *55*(3), 233-240.

National Farm Animal Care Council. (2012) Recommended code of practice for the care and handling of farm animals: pigs. Ottawa, ON, Canada: AAFC Publication.

National Research Council. Nutrient requirements of swine. (2012) 11th rev. ed.Washington, DC, USA: *The National Academies Press*.

Oliveira, E. B. D., Gotschlich, C., & Liu, T. Y. (1979). Primary structure of human C-reactive protein. *Journal of Biological Chemistry*, *254*(2), 489-502.

Ominski, K. H., McAllister, T. A., & McKinnon, J. J. (2021). Sustainable pig production: Challenges and opportunities. *Animal Feed Science and Technology*, 271, 114697.

Pomar, C., Hauschild, L., Zhang, G. H., Pomar, J., & Lovatto, P. A. (2011). Precision feeding can significantly reduce feeding cost and nutrient excretion in growing animals. In *Modelling nutrient digestion and utilisation in farm ani*mals (pp. 327-334). Wageningen Academic Publishers, Wageningen.

Pomar, C., Gonzalez, J. M., & Nogueira, J. C. (2019). Precision feeding in pigs: Strategies and benefits. *Animal Feed Science and Technology*, 253, 1-14.

Remus, A., Hauschild, L., & Pomar, C. (2019a). Simulated amino acid requirements of growing pigs differ between current factorial methods. *Animal*, 14(4), 725-730.

Remus, A., C. Pomar, D. Perondi, J. P. Gobi, W. C. da Silva, L. J. de Souza, and L. Hauschild. (2019b). Response to dietary methionine supply of growing pigs fed daily tailored diets or fed according to a conventional phase feeding system. *Livestock Science*, 222:7-13.

Remus, A., L. Hauschild, E. Corrent, M.-P. Létourneau-Montminy, and C. Pomar. (2019c). Pigs receiving daily tailored diets using precision-feeding techniques have different threonine requirements than pigs fed in conventional phase-feeding systems. *Journal of Animal Science and Biotechnology*, 10(1):16.

Remus, A., Hauschild, L., Létourneau-Montminy, M. P., Corrent, E., & Pomar, C. (2020). The ideal protein profile for late-finishing pigs in precision feeding systems: threonine. *Animal Feed Science and Technology*, *265*, 114500.

Santos, L. S. D., Pomar, C., Campos, P. H. R. F., da Silva, W. C., Gobi, J. D. P., Veira, A. M., ... & Hauschild, L. (2018). Precision feeding strategy for growing pigs under heat stress conditions. *Journal of Animal Science*, 96(11), 4789-4801.

Soto, J., González, J., & Muñoz, J. (2019). Impact of sanitary challenges on amino acid requirements and growth in pigs. *Veterinary Journal*, 246, 40-47.

van Milgen, J., Valancogne, A., Dubois, S., Dourmad, J. Y., Sève, B., & Noblet, J. (2008). InraPorc: a model and decision support tool for the nutrition of growing pigs. *Animal Feed Science and Technology*, 143(1-4), 387-405.

van Milgen, J., & Dourmad, J. Y. (2015). Concept and application of ideal protein for pigs. *Journal of Animal Science and Biotechnology*, 6(1), 1-11.

CAPÍTULO 4 – Discussão geral

4.1. Principais resultados, perspectivas e conclusões

4.1.1. Sistema de alimentação de precisão

O IPF utiliza o histórico de peso corporal e consumo de cada indivíduo para projetar o seu crescimento e determinar a concentração dietética que irá suportar o desempenho estimado. Essa metodologia foi desenvolvida para possibilitar a alimentação precisa de suínos considerando a variação de desempenho entre indivíduos. A utilização de outras técnicas para estimar o desempenho, como a adoção de curvas de crescimento pré-estabelecidas, assume que o desempenho dos indivíduos será semelhante ao do animal médio e desconsidera a variabilidade da população. Estudos anteriores demonstraram os benefícios da aplicação do IPF na redução do fornecimento de Lys e excreção de N sem comprometer o desempenho (Andretta et al., 2014 e 2016; Santos et al., 2018).

No entanto, os dados do presente estudo demonstram que crescimento dos suínos não desafiados foi prejudicado quando alimentados com o IPF. A figura abaixo demonstra o consumo de ração diário dos suínos antes e depois do início do período experimental (Figura 1). Embora menos expressiva do que nos animais desafiados, houve redução no consumo dos animais não desafiados durante a primeira semana experimental. De maneira similar, houve redução no ganho de peso diário dos animais não desafiados (dados não apresentados). Por predizer exigências futuras através do histórico de consumo e crescimento, é possível que o IPF tenha subestimado o desempenho dos animais após a primeira semana. Consequentemente, a quantidade de nutrientes fornecida diminuiu, o que pode ter limitado o crescimento dos animais.

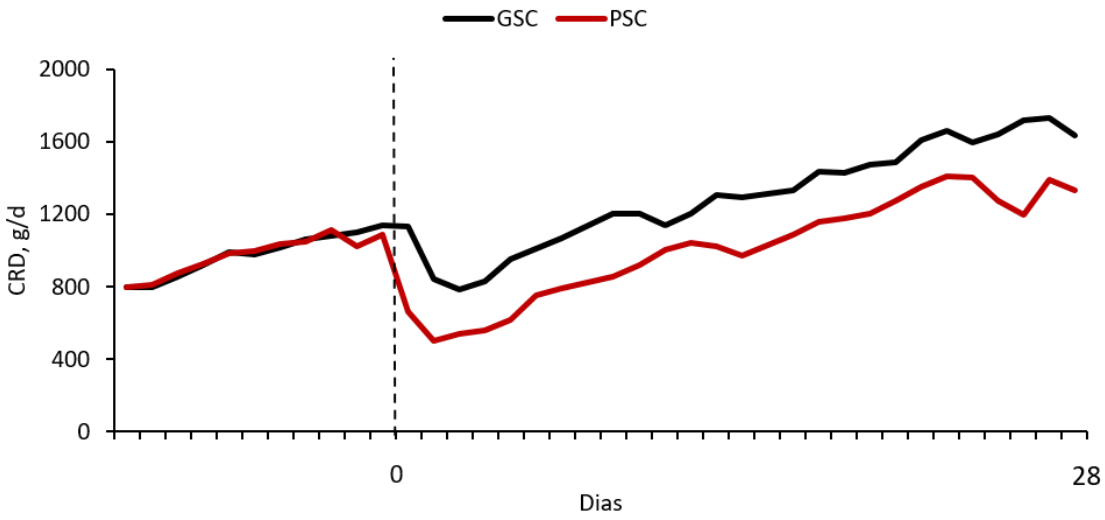

Figura1. Consumo de ração diário de suínos alojados em condições sanitárias boas (GSC) e ruins (PSC) ao longo do período experimental. A GSC foi estabelecida com rígido protocolo de biosseguridade. A PSC foi estabelecida através da inoculação oral de 2 × 10^9 ufc de *Salmonella* Typhimurium e da aplicação de fezes de uma granja comercial de suínos no chão da baia.

É importante salientar que a parte mecanicista do modelo possui um componente de suavização que ameniza o impacto das variações no consumo e peso corporal observados na predição do PC e CRD (Hauschild et al., 2012). No entanto, este componente não evitou que as variações de desempenho interferissem nas estimativas de exigência de Lys. Os dados observados sugerem que pesquisas futuras sejam desenvolvidas no intuito de ajustar as exigências estimadas em situações de redução no PC e CRD não atrelados ao desafio sanitário.

4.1.2. Sistema de alimentação de precisão para suínos submetidos ao desafio sanitário

O sistema de alimentação de precisão prejudicou o desempenho de suínos quando estes foram submetidos ao desafio sanitário. Além disso, concordando com estudos anteriores, o IPF reduziu o fornecimento de N e Lys. Entretanto, não houve aumento da eficiência de utilização dos mesmos. Esses dados indicam que o atual modelo do IPF limitou o desempenho de suínos desafiados. O desafio

imune modifica o metabolismo dos animais direcionando a fisiologia e nutrientes para o suporte da resposta imune. Essas modificações alteram a exigência de mantença dos animais além de diminuir o seu potencial de deposição de proteína. Portanto, alimentar animais desafiados avaliando apenas o seu crescimento e desconsiderando as alterações fisiológicas decorrentes da ativação do sistema imune pode resultar no fornecimento de dietas com concentrações abaixo daquelas requeridas.

Para superar as limitações do IPF observadas neste estudo, torna-se essencial o desenvolvimento de técnicas de modelagem avançadas que permitam estimar com precisão as exigências nutricionais de suínos desafiados. A aplicação dessas técnicas possibilitaria ajustar as exigências considerando os efeitos do sistema imunológico sobre as necessidades de mantença e crescimento dos animais. No entanto, a intensidade com que o desafio imunológico afeta o crescimento dos suínos está relacionada a uma miríade de fatores, como o tipo de patógeno, a dose e as características do hospedeiro (Sandberg et al., 2007). Além da escassez de estudos que avaliem esses fatores, as interações entre o sistema imunológico e o metabolismo animal são complexas e ainda pouco compreendidas. Nesse contexto, a determinação precisa das exigências nutricionais é desafiadora, especialmente quando se busca formular dietas com menor excesso de nutrientes, como no IPF. Apesar dessas dificuldades, compreender as alterações metabólicas e definir as exigências nutricionais de suínos desafiados é fundamental para aumentar a acurácia com a qual alimentamos os animais no IPF.

4.1.3. Suplementação de aminoácidos funcionais

Não foram observados efeitos da suplementação de AA funcionais em nenhuma variável analisada. Isso indica que, para as condições experimentais aqui utilizadas, a suplementação de AA funcionais não atenuou os efeitos negativos associados a ativação do sistema imunológico. A maioria dos estudos que avaliaram a suplementação de Met, Thr e Trp para suínos desafiados, utilizou as relações AA:Lys sugeridas pelo NRC (2012) (van der Meer et al., 2016;

Rodrigues et al., 2023; Valini et al., 2024). As relações de Met, Thr e Trp utilizadas no Capítulo 2 são 7%, 8% e 15% maiores do que as do NRC. Isso sugere que as relações de AA utilizadas na dieta controle possam ter suprido a demanda de AA do sistema imune.

Finalmente, durante o período experimental de 28 dias, os suínos alimentados com dietas suplementadas ingeriram mais aminoácidos funcionais em comparação com aqueles que receberam a dieta controle. No entanto, durante a primeira semana de desafio, tanto os suínos alimentados com a dieta suplementada quanto os alimentados com a dieta controle consumiram quantidades semelhantes de aminoácidos funcionais. Esses resultados sugerem que a ausência de diferenças significativas entre as dietas pode estar relacionada à similaridade na ingestão de aminoácidos durante a fase aguda da ativação imunológica. Assim, o benefício da suplementação de aminoácidos funcionais parece estar intrinsecamente ligado às relações de AA utilizadas na formulação das dietas, à intensidade da ativação do sistema imunológico e ao consumo total de aminoácidos durante o período de desafio. Isso reforça a necessidade de uma abordagem mais refinada na formulação de dietas que leve em conta esses fatores para maximizar os efeitos positivos da suplementação em condições de desafio sanitário.

4.1.4. Relações de amino ácidos para suínos com diferentes taxas de crescimento

Os resultados desta tese revelam que a manipulação das relações de AA para suínos com diferentes taxas de crescimento exerce influência significativa sobre o desempenho e a composição corporal dos animais. Em suínos alimentados com alimentação de precisão, observou-se que o aumento das relações de Thr foi particularmente benéfico para suínos de crescimento lento, resultando em um aumento linear do ganho diário de peso e melhorando a eficiência da deposição proteica. Essa resposta sugere que suínos de crescimento lento podem necessitar de níveis mais elevados de aminoácidos funcionais para otimizar a deposição de proteína corporal, enquanto suínos de crescimento mais

rápido podem não se beneficiar tanto dessas elevações, uma vez que seu desempenho já se encontra em um nível elevado mesmo com dietas com menor concentração de AA. Esses achados são relevantes para a formulação de dietas no sistema de alimentação de precisão, pois mostram que a taxa de crescimento deve ser considerada ao determinar as relações AA.

Além disso, os resultados desta tese apontam para a conclusão de que conceitos tradicionais, como proteína ideal, podem não ser os mais adequados para a formulação de dietas de alimentação de precisão. Portanto, há uma necessidade de explorar e desenvolver novos métodos para estimar as necessidades individuais de AA que sejam mais responsivos à natureza dinâmica da alimentação de precisão. Pesquisas futuras devem focar no aprimoramento dessas técnicas de estimativa, possivelmente integrando dados de crescimento em tempo real e abordagens avançadas de modelagem, para formular dietas mais adequadas às necessidades específicas de cada animal. Isso poderia levar a estratégias de alimentação mais precisas que podem melhorar a eficiência de produção.

4.2. Conclusões da tese

Esta tese demonstra que o aumento das relações de aminoácidos funcionais em suínos desafiados oferece benefícios que são altamente dependentes da intensidade do desafio, das relações de AA utilizadas, e da ingestão alimentar. A pesquisa destaca que o sistema atual de alimentação de precisão, ao estimar as exigências individuais, pode subestimar as necessidades de suínos em condições de desafio sanitário. Isso indica a necessidade de novas abordagens para a estimativa de exigências nutricionais, especificamente voltadas para suínos sob condições de desafio, com o objetivo de otimizar a aplicação do IPF.

Além disso, a tese mostra que as relações ótimas de Thr:Lys para maximizar o desempenho de suínos no IPF são influenciadas pela taxa de crescimento dos animais. Isso sugere que a taxa de crescimento deve ser um critério chave na formulação de dietas no IPF, permitindo uma abordagem mais personalizada e eficiente na alimentação dos suínos.

Por fim, a tese sublinha a importância de aperfeiçoar o sistema de alimentação de precisão, identificando áreas de melhoria que possam aumentar a eficácia e precisão com que os suínos são alimentados. Isso inclui o desenvolvimento de novas técnicas de modelagem avançadas que incorporem dados de crescimento e *status* sanitário em tempo real, possibilitando uma nutrição mais adaptada às necessidades específicas de cada animal e, consequentemente, uma produção mais eficiente.

4.3. Referências

Andretta, I., Pomar, C., Rivest, J., Pomar, J., Lovatto, P. A., & Radünz Neto, J. (2014). The impact of feeding growing–finishing pigs with daily tailored diets using precision feeding techniques on animal performance, nutrient utilization, and body and carcass composition. **Journal of Animal Science**, 92(9), 3925-3936.

Andretta, I., Pomar, C., Rivest, J., Pomar, J., & Radünz, J. (2016). Precision feeding can significantly reduce lysine intake and nitrogen excretion without compromising the performance of growing pigs. **Animal**, 10(7), 1137-1147.

Delmastro-Greenwood, M. M., & Piganelli, J. D. (2013). Changing the energy of an immune response. **American Journal of Clinical and Experimental Immunology**, *2*(1), 30.

Hauschild, L., Lovatto, P. A., Pomar, J., & Pomar, C. (2012). Development of sustainable precision farming systems for swine: estimating real-time individual amino acid requirements in growing-finishing pigs. **Journal of Animal Science**, 90(7), 2255-2263.

National Research Council. (2012). Nutrient requirements of swine.

Ortiz, M. T., Arnaut, P. R., Valini, G. A. C., França, I., Silva, C. A., de Oliveira, M. J. K., ... & Hauschild, L. (2024). A salmonella-challenge impacts the variability of performance, body composition and lysine requirements of growing pigs under poor housing conditions. **Livestock Science**, 105462.

Pastorelli, H., Van Milgen, J., Lovatto, P., & Montagne, L. (2012). Meta-analysis of feed intake and growth responses of growing pigs after a sanitary challenge. **Animal**, 6(6), 952-961.

Rodrigues, L. A., Wellington, M. O., González-Vega, J. C., Htoo, J. K., Van Kessel, A. G., & Columbus, D. A. (2021). Functional amino acid supplementation, regardless of dietary protein content, improves growth performance and immune status of weaned pigs challenged with Salmonella Typhimurium. **Journal of Animal Science**, 99(2), skaa365.

Sandberg, F. B., Emmans, G. C., & Kyriazakis, I. (2007). The effects of pathogen challenges on the performance of naïve and immune animals: the problem of prediction. **Animal**, 1(1), 67-86.

Santos, L. S. D., Pomar, C., Campos, P. H. R. F., da Silva, W. C., Gobi, J. D. P., Veira, A. M., ... & Hauschild, L. (2018). Precision feeding strategy for growing pigs under heat stress conditions. **Journal of Animal Science**, 96(11), 4789-4801.

Valini, G. A. C., Arnaut, P. R., França, I., Ortiz, M. T., Oliveira, M. J. K., Melo, A. D. B., ... & Hauschild, L. (2023). Increased dietary Trp, Thr, and Met supplementation improves growth performance and protein deposition of salmonella-challenged growing pigs under poor housing conditions. **Journal of Animal Science**, 101, skad141.

Van der Meer, Y., Lammers, A., Jansman, A. J. M., Rijnen, M. M. J. A., Hendriks, W. H., & Gerrits, W. J. J. (2016). Performance of pigs kept under different sanitary conditions affected by protein intake and amino acid supplementation. **Journal of Animal Science**, 94(11), 4704-4719.

Van der Peet-Schwering, C. M. C., Koopmans, S. J., & Jansman, A. J. M. (2019). Amino acid requirements in relation to health status in growing and finishing pigs (No. 1168). **Wageningen Livestock Research**.