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Par

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In silico and *in vitro* approaches to investigate small intestinal metabolism of amino acids

Approches *in silico* et *in vitro* pour investiguer le métabolisme des acides aminés dans l'intestin grêle

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Composition du Jury :

Président :	Didier Rémond	Directeur de recherche, UNH, INRAE
Examinateurs :	Amélie Deglaire	Maîtresse de conférences, Institut Agro Rennes-Angers
	Rafael Muñoz-Tamayo	Directeur de recherche, UMR MoSAR, INRAE
Dir. de thèse :	Jaap van Milgen	Ingénieur de recherche, UMR PEGASE, INRAE
Co-dir. de thèse :	Nathalie Le Floc'h	Directrice de recherche, UMR PEGASE, INRAE
	Yves Mercier	Global Technical & Scientific Support Manager, Adisseo

Remerciements

La rédaction de ces remerciements ayant été à plusieurs reprises repoussée, je me décide enfin à me consacrer à cette touche finale du travail de thèse. Touche finale qui n'est pas des moindre, car c'est celle qui sera la plus lu ! On attend de manière classique que les remerciements permettent de témoigner de la reconnaissance à quelconque individu ayant contribuer au projet. Mais, la rédaction des remerciements est aussi pour l'auteur (moi-même) une étape importante puisqu'elle permet de clore définitivement ce travail, et surtout de prendre de la perspective sur ces trois années. C'est l'occasion de mettre en ordre les ressentis qui s'accumulent, d'émulation ou de fierté mais aussi de déception parfois – ainsi de prendre la mesure du relief de cette période. La lecture des remerciements est aussi, pour toute personne extérieur au projet, le moyen de saisir le contexte dans lequel s'est déroulé le travail. De chercher à comprendre qui à écris ce travail, comment il a été vécu, avec quelles collaborations… En tous cas, c'est avec cette curiosité que je lis les remerciements d'une thèse ! Bref, c'est tous ces vécus que l'on ne peut pas inclure dans un article scientifique qui sont mis à l'honneur ici. Parfois pendant la thèse, j'en ai imaginé certains passages, des anecdotes à inclure ou des tournures de phrase. Me voici maintenant face à mon clavier et un document texte vide (un document Open Office), que je m'apprête à remplir. Pour y rassembler des idées éparses sous la forme d'un texte - aussi structuré que possible.

Je tiens d'abord à remercier mon jury, qui a accepté d'évaluer ce travail de thèse. Je remercie donc mes deux rapporteurs Linda Giblin et Walter Gerrits ainsi que mes trois examinateurs, Rafael Muñoz-Tamayo, Amélie Deglaire et Didier Rémond. J'attendais avec hâte leurs retours sur mon travail de thèse, car c'était la première fois depuis le début de qu'il était évalué dans son ensemble. Je les remercie donc pour nos échanges pendant la soutenance, qui fut un moment très riche pour moi. C'était un moment privilégié pour moi que d'avoir « à ma disposition » les avis et les remarques d'un panel de scientifiques, apportant chacun un regard différent sur cette thèse.

Évidemment, je souhaite remercier mes trois encadrants, qui m'ont suivi pendant ces trois années de thèse. Tout d'abord Jaap, qui était le directeur de cette thèse et qui gardait dans un coin de sa tête l'idée initiale de ce projet de thèse depuis quelques années. Je me souviens de notre première entrevue en visio alors que j'étais aux Pays-Bas, au sujet du stage, qui a donné

suite à cette thèse. Durant ce premier échange, j'avais déjà pu mesurer ton enthousiasme pour la recherche ainsi que la curiosité insatiable qui t'animes. J'ajouterai que c'est aussi une forme de créativité chez toi qui m'a marqué, car à toutes nos réunions tu avais de nouvelles idées, des choses à tester en modélisation ou un nouveau point de discussion à ajouter dans un article. Durant ces presque 4 années, si l'on compte le stage et le CDD, nous avons fonctionné avec beaucoup d'autonomie, ce qui m'allait très bien. Ce mode de fonctionnement m'a permis d'avoir un large espace d'expression dans mon travail, tout en ayant un appui quand j'en avais besoin. Le mot que je retiendrais de ces années de travail avec toi, sera la simplicité. Car face à des sujets de recherches complexes et la tentation de partir dans de multiples directions, le choix le plus simple est souvent le meilleur.

Je remercie Nathalie d'avoir co-encadrée cette thèse. Merci d'avoir apporté à ce projet ta rigueur scientifique, qui m'a poussée à clarifier mes idées et à justifier mes choix méthodologiques. Je garderai de mon travail avec toi cette exigence, que ce soit dans la rédaction ou l'interprétation de données. Ton regard de « non modélisatrice » m'a aussi permis de prendre du recul sur mon travail. Je te remercie aussi pour la touche d'empathie que tu as apporté dans cet encadrement. Ta capacité d'écoute active a fait que je suis souvent venu vers toi quand j'avais besoin d'exprimer des difficultés au sujet de la thèse. Je retiendrais nos longues discussions dans ton bureau, qui commençaient par le trajet en vélo du jour et qui finissaient sur de vastes sujets d'écologie ou de société !

Enfin, je te remercie Yves d'avoir encadré cette thèse du côté d'Adisseo, à distance. Tu as suivi le rythme tout en étant loin (Commentry ça n'est pas à coté). Tu m'as aussi fait confiance pour un sujet de recherche assez original, et parfois loin de tes travaux habituels. Merci aussi pour tes conseils tout au long de la thèse et ta disponibilité quand j'en avais besoin.

Je veux aussi remercier Adisseo, qui a co-financé cette thèse par un dispositif Cifre. Je n'ai certes pas été beaucoup présent au sein de l'entreprise, du fait de la distance. Mais j'ai toujours eu affaire à des personnes accueillantes lors de mes quelques séjours à St-Fons ou Commentry. A mon arrivée, le médecin du travail, lors de la visite médicale m'a dit que j'étais tombé dans une entreprise bienveillante. Je peux dire que c'est le cas.

Je souhaite aussi remercier les membres de mon CSI, Steven Le Feunteun, Gaëlle Boudry et Bertrand Méda. Les réunions de CSI ont toujours été des moments riches pour moi, pour faire

le point sur la situation et se projeter vers la suite. Vos conseils m'ont beaucoup aidé à m'orienter et à prendre confiance, surtout suite au premier CSI. Je vous remercie donc pour la pertinence et la bienveillance de vos remarques !

Après une première série de remerciements je souhaite dire quelques mots sur la genèse de ce projet. J'ai mis les pieds pour la 1ère fois à PEGASE en mars 2021, à une période où l'on portait des masques, on mangeait isolé à la cantine et l'on rentrait chez soit le soir avant le couvre feu. C'était pour un stage de fin d'études de 6 mois, déjà pour travailler sur les acides aminés dans l'intestin, par méta-analyse et modélisation. C'est au cours de ce stage que l'idée de continuer avec une thèse a germée. Une fois l'idée lancée, il a fallu trouver un potentiel financement, qui a été le dispositif Cifre avec Adisseo. Par la suite, le projet a été rédigé et envoyé à l'ANRT pour obtention de la bourse Cifre alors que j'étais en CDD toujours à PEGASE, puis il a fallu attendre la réponse. Au début 2022, les mois m'ont paru long lors de cette attente, où j'étais bien peu occupé. La réponse de l'ANRT est finalement arrivée début mai, pour un début de la thèse au 1er juin. Je me souviens bien de ce mois de mai 2022, agréable puisque j'étais en vacances pour solder ce qui me restait de congés, avec une semaine de congrès DPP à Rotterdam et l'excitation de la thèse à venir !

Une fois la thèse lancée, je me suis mis pleinement dans les travaux de modélisation qui ont avancés plus vite que je ne le pensais. A ce moment de la thèse, l'objectif était d'utiliser les trois ans de thèse pour développer un modèle conceptuel d'intestin, qui serait progressivement amélioré au cours du temps. Ce travail très conceptuel de modélisation m'interrogeait parfois et je manquais d'avancées concrètes dans mon quotidien. C'est donc en ce début de thèse que l'idée de compléter la modélisation par une approche expérimentale a germée. Le travail de modélisation me laissait suffisamment de temps pour mener en parallèle une autre approche. C'est au moment du premier CSI, après 6 mois de thèse que s'est concrétisée l'ajout du volet culture cellulaire à la thèse. L'objectif était d'obtenir des données et des lois de réponses pour l'absorption d'acides aminés par les cellules intestinales, « i*n vitro* ». La visée de cet ajout pour moi était double, tout autant de complémenter l'approche scientifique de la thèse, que de développer des compétences nouvelles.

Avec le recul, je me dis que cet ajout était ambitieux, puisque ni moi ni mes encadrants n'avions d'expertise en culture de cellules. En plus, nous avions décidé d'utiliser un modèle cellulaire

(IPEC-J2) encore peu maîtrisé, dont la culture venait d'être lancée à PEGASE. Mais, l'idée était prometteuse et surtout originale !

Ici, je remercie Marie-Hélène Perruchot, qui m'a aidé à me lancer dans la culture de cellules. C'est grâce à tes conseils que j'ai appris (en partant de zéro) les bases de culture des cellules, la désinfection du matériel, le changement de milieux, le comptage...etc. Merci d'avoir était une bonne prof' de culture cellulaire ! Ce fut donc le début d'un long travail de développement de méthode, qui commençait par la maîtrise de la culture des cellules, avant de s'attaquer à l'utilisation de marquages isotopiques – mais j'y reviendrai.

De leurs côté, les travaux de modélisation ont bien avancé, et après 1 an et demi, je pouvais soumettre mon 1er article à un journal. C'est étonnant comme le dernier mois de travail de modélisation ont portés sur des petits détails, qui paraissaient peu signifiants mais qui à chaque fois demandaient un réajustement de paramètres, de nouvelles simulations… Bref, chaque hypothèse du modèle a été discuté et testée avant la soumission, dans l'idée d'être que chaque partie pris soit cohérent. Dans l'ensemble, le travail de rédaction d'article m'a plu. Le fait de choisir les éléments que l'on veut mettre en valeur et discuter au regard de la littérature scientifique est selon moi la partie la plus intéressante du travail de recherche. C'est la partie où l'on peut le plus exprimer sa créativité, l'originalité de ses idées. Le travail de modélisation qui avait été mené ici était d'ailleurs très adapté à servir de support à une discussion sur portée sur les concepts et les mécanismes biologiques sous-jacents. Bref, après avoir rédigé l'article, il a fallu le soumettre et se préparer à un long chemin vers la publication, qui sans être sinueux, a pris une année entière.

Du côté de la culture cellulaire, l'avancée était moins évidente et je me heurtais à des difficultés méthodologiques. L'utilisation de cellules intestinales cultivées au laboratoire, avec des isotopes stables étant nouvelle, il fallait passer par une étape de mise au point. Et il s'avère que rien qu'obtenir des conditions de culture à stables ne fut pas aisé. L'ajustement du volet isotopie, avec le marquage des acides aminés non plus d'ailleurs !

Je garde tout de même de très bons souvenirs de mes journées en salle de culture cellulaire ! Cette petite salle isolée au fond un sous-sol ne paie pas de mine comme ça. Mais il y règne un niveau de bonne humeur plus élevé que dans la moyenne des autres laboratoires. J'en profite pour remercier Fred D., Fred M., et Laurence F. que j'ai rencontré « là-bas » ! J'ai beaucoup

apprécié l'ambiance de votre petite équipe, et cette touche de rigolade dans le travail. Surtout avec ce « pitre » de Fred D., dont je sais que les nombreuses blagues sur ma chevelure cachaient une jalousie profonde (désolé Fred...).

La partie du isotopie de ce travail a mené à une collaboration avec l'IRHS à Angers – en tant que spécialistes du sujet. Je fus guidé par Guillaume Tcherkez et Julie Lalande, qui m'ont initié aux subtilité de l'utilisation d'isotope stables. Je tiens à vous remercier tous les deux pour votre implication dans le projet. Même en tant que non spécialistes des acides aminés dans les cellules animales, j'ai toujours été impressionné par votre capacité à enrichir le projet de nouvelles suggestions à chaque réunion. Je n'oublie pas aussi de remercier Jean-Baptiste Domergue, pour son aide à la préparation des échantillons.

Une fois l'expérimentation en culture cellulaire faite à St-Gilles, j'allais donc à Angers avec un sac chargé d'échantillons à préparer pour les faire analyse en IRMS. Je crois que j'ai eu au total 6 séjours de plusieurs jours à Angers à chaque fois pour les préparations d'échantillons. J'ai beaucoup apprécié ces petites escapades Angevines, qui étaient l'occasion de changer d'atmosphère de travail et de revoir quelques copains au passage, dans cette si douce ville ! Le travail de préparation d'échantillons en soit consistait beaucoup à la peser des échantillons à la balance micrométrique. C'est à dire, à peser 1 mg (± 10 %) de poudre par échantillon, qu'il fallait déposer délicatement dans une petite capsule en métal. Autant dire, un travail de haute précision (dont je ne me saurai cru capable). L'apprentissage de cet art commença par une vitesse moyenne par un rendement horaire de travail beaucoup trop faible (25 min/échantillon, alors que j'en avais 50) et la contamination de la balance au ^{13}C (oups). A force de pratique et de patience, j'ai appris à enchaîner les pesées à un rythme élevé, en plaçant du 1er coup la bonne quantité dans la capsule (mon œil s'est affûté pour estimer 1 mg de poudre à vue). J'occupais donc mes journées à faire des pesées, tout en écoutant des podcasts. J'ai une pensée particulière pour les séries « Affaire sensible », « Les naufragés » et « Face à l'histoire », de Radio France, qui furent des compagnons privilégiés. Quelle chance tout de même, que le service public nous propose des divertissements d'une telle qualité – et ce gratuitement !

Tous ces travaux en culture cellulaire et isotopie ont donc amenés à des 1ers résultats intéressants. Évidemment, ces résultats sont moins avancés que je ne l'aurais espéré au lancement de ce volet de la thèse. Mais nous avions sous estimé la complexité de ce type d'approche, surtout en

sachant que ce travail incluait tout une partie de mise au point de méthode. Néanmoins, ces premiers résultats sont originaux, surtout dans l'optique de les associer au travail de modélisation réalisé en parallèle dans la thèse. J'espère donc qu'ils pourront être valorisés dans un journal, pour aller au bout du processus de production scientifique et les rendre accessibles.

De son coté, le travail de modélisation a bien continué sur sa lancée. Le modèle créé initialement et valorisé dans un 1er article, a été amélioré pour inclure de nouveaux aspects. De nouvelles simulations ont pu être faites, pour le tester dans des situations variées. Un second article a ainsi pu être écris, qui à l'heure où j'écris ces mots, est soumis à un journal !

Le travail n'est pas que l'action de produire des biens et services, mais aussi un lieu de socialisation pour les individus. Cette socialisation s'est parfois réalisée au détour d'une rencontre dans un couloir, lors d'une discussion dans un bureau ou encore pendant les dites pauses. On dénombre principalement deux pauses dans une journée de travail : la pause de 10h30 ou « pause café », et la pause de midi ou pause méridienne. Cette dernière, est dédiée à la consommation d'un repas, qui permet de passer la deuxième partie de la journée sereine. Je tiens ici à remercier les principaux artisans de la réussite de cette pause : Wilfried et Nina. Merci à vous deux pour votre bonne humeur chaque jour et ces plats si bons ! Combien de travaux de recherche menés ici dans cette unité n'iraient nulle part sans vous ? Je n'ose pas compter. En tous cas, pour moi, ce fut un plaisir de vous revoir chaque midi et vous allez me manquer !

J'ai aussi une pensée (émue) pour l'ensemble de l'équipe « contrats précaires ». Une équipe de recherche à part entière, malgré sa non reconnaissance auprès de la direction de l'unité. Tous ces moments partagés dans le cadre du travail, que ce soit les pauses, les congrès, les discussions en fin de journée… Mais aussi les nombreuses sorties en extérieur, au bar, à des courses à pied, un week-end à Paris, du vélo dans le Cotentin (<3)… Ces liens ont été très importants pour moi, pour rendre mon cadre de travail agréable. La preuve de l'importance de ces liens est que l'on continue de voir avec bon nombre d'entre vous, malgré les départs de chacun vers de nouveaux horizons. Évidemment, le bureau 84 fut l'épicentre de nos réunions. Ce bureau a l'ambiance si réputée et qui pouvait se vanter de faire couler le meilleur café de tout Pegase.

Je pense ici aux plus anciens, les vieux briscards du bureau 84: Lorry (j'envie toujours ta foulée si véloce), Elise (notre Cassandre Beaugrand à nous), Emma (merci pour tout et surtout de

m’avoir fait découvrir le baume du tigre), Ellynn (qu’est-ce que tu deviens Eline ?), JC (par pudeur, je n’ose trop écrire ici, mais j’ai tant d’images en tête...), Angélique (à la détermination sans faille), Alyson (à l’étoile filante sudiste que tu as été dans nos vies). Mais aussi à celles et ceux qui sont arrivées plus tard : Clemente (c’était un plaisir de partager une identité commune de Cléments ensemble pendant ces 3 ans), Emmanuelle (promis, un jour je viendrai à St-Gilles, quoiqu’il arrive), Chloé (pour avoir amené une touche de Belgique jusqu’ici), Auxane (j’ai apprécié t’accueillir dans mon bureau à l’Agro ! Je te laisse le droit de continuer à l’utiliser, en mon souvenir.), Coralie (amuse toi bien avec Python). Mais aussi tous les autres que j’ai côtoyé pendant ces 3 (ou 4) années : Mariana, Francis, Aira, Léa, Estelle, Dimitri, Ilona, Alicia, Camille, Adrien, Alexandre…

Sur la dernière année de thèse, j’ai profité de la rédaction pour passer plusieurs jours par semaine à l’Agro. J’y ai trouvé un cadre de travail agréable et - atout non négligeable - à proximité de mon domicile. Je remercie toute l’équipe du bâtiment 15 à l’Agro, pour leur accueille ! Étant expatrié depuis St-Gilles, je me suis très vite senti parfaitement intégré ici. Merci de m’avoir introduit à votre activité favorite : les mots croisés à la pause de 10h30. Mais aussi merci de m’avoir introduit à votre seconde activité favorite : les mots croisés à la pause du midi. Je pense avoir progressé, même si je suis bien loin du (très) haut niveau qui est atteint ici. En tous cas, c’était toujours un plaisir passer des moments en votre compagnie en cette dernière année !

Je tiens à remercier ma cheffe d’équipe Florence Garcia, pour l’accueil. J’ai particulièrement apprécié ton approche participative de la gestion d’équipe et les initiatives que tu as mis en place pour améliorer les réunions d’équipe.

Je veux aussi remercier Myriam Grundy pour tous les échanges que nous avons eu. Nous sommes arrivés presque en même temps à Pegase, je me souviens que nous avons sympathisé lors d’un cours à Wageningen. J’ai apprécié lors de nos discussions ta réflexion sur ton métier de chercheuse et ton approche novatrice de ta thématique de recherche. Mais aussi nos discussions sur des sujets tels que l’écologie, le vélo et bien d’autres. Je pense qu’on se recroisera, au marché des Lys ou ailleurs !

J’ai aussi une pensée pour mon collègue de bureau, Romain. Nous avons partagé ce petit bureau, dont nous avons été les premiers pensionnaires ensemble. Je te souhaite plein de bonnes choses pour tes nouvelles missions à l’INRAE !

Un petit mot aussi pour ma voisine, dans son laboratoire d’a côté, Colette. J’ai beaucoup aimé ce pas de côté que tu amenais par ta spontanéité, ton recul sur le travail. J’espère qu’on se recroisera, à d’autres occasions !

Pendant ces trois années de thèse, j’ai eu l’occasion de m’impliquer au sein de l’école doctorale. Je remercie et je salue Yannick Outreman et Isabelle Baumgarten pour leur implication au service des doctorants.

J’ai aussi une pensée pour toutes les personnes que j’ai rencontré à Wageningen aux Pays-Bas, juste avant d’arriver à Pegase. Ces deux années de master ont été une excellente préparation à la thèse. Sonja, thank you for introducing me to the scientific activity during my thesis. This first experience taught me a lot of useful things that I later used during the PhD. I particularly remember the first drafts of texts I sent to you, which came back all red from your review – I think I remembered the lesson! Je salue aussi l’équipe des Français de Wageningen, Corentin, Gauthier, Martin, François. Tous ces footings, les verres à Onder de Linden, les debriefs du samedi midi autour des kibblings du marché, nos discussions politico-scientifiques, les repas, les j... On s’est bien amusé, malgré le covid ! Je suis heureux d’avoir réussi à garder le contact au cours de ces 3 années de thèse.

Avec le recul, je dirai que la rédaction de la thèse fut la partie du travail de thèse que j’ai trouvé la plus intéressante. J’ai aimé écrire la revue de littérature, que s’est retrouvée plus longue que prévue. Moi qui voulais faire le plus court possible, je me suis retrouvé à écrire 50 pages sur les éléments que je trouvais « essentiels » à la contextualisation du sujet. Il ne faut pas trop penser au fait qu’à par le jury, personne ne lira ces 50 pages, mais garder l’intérêt de l’exercice pour soi ! La rédaction de la discussion générale fut aussi un exercice agréable, car très libre. J’ai pu partir des résultats de ma thèse pour élaborer d’abord sur des éléments concrets - liés au sujet - puis sur des réflexions plus larges. Je suis persuadé qu’inclure des réflexions globales dans une discussion de thèse a son intérêt. Puisqu’il est attendu d’un chercheur d’être capable de contextualiser sa recherche et de prendre de la perspective sur les travaux menés. Surtout quand le titre décerné s’intitule en anglais « Philosophy Doctor (PhD) ». Un ami avait un jour affirmé

que la partie philosophie d'une thèse de science est souvent négligée. C'est une réflexion que je partage.

Finalement, toutes ces 3 années de travail se concluent par cette thèse, qui est contenue dans ce document. C'était un projet de recherche ambitieux, pluridisciplinaire et je suis heureux d'avoir été au bout. Trois ans de thèse, cela m'a permis de développer des compétences, directement sur mon sujet, par la lecture d'articles, les conférences. Mais aussi plus techniques, pour moi la culture cellulaire, l'isotopie et la modélisation. Et enfin, des compétences transversales, de rédaction scientifique, présentation orale, gestion de projet...etc. Bref, je peux dire que oui, j'ai appris beaucoup de choses pendant ces 3 années de thèse ! Et puis, il y a la production scientifique issue de ces travaux. C'est un apport, certes modeste, à la compréhension des flux d'acides aminés dans l'intestin. Ainsi qu'à l'utilisation de méthodes alternatives à l'expérimentation animale pour étudier la nutrition.

Maintenant, se pose la question mon ressenti personnel par rapport à ce travail de thèse. Je ne cache pas que j'ai souvent douté pendant les trois ans, et souvent de la pertinence de mon travail de recherche. Même si j'ai aimé l'univers de la recherche et l'exercice qu'était la thèse, je n'ai pas eu de rapport passionnel à mon sujet. Je n'ai pas accroché au projet scientifique de mon équipe, ce qui a vite limité les stimulations dans mon environnement de travail. J'ai d'ailleurs exprimé mes différences d'opinion sur certains axes de recherche développés dans l'équipe, dans la discussion générale de cette thèse Et puis, le travail de thèse est très solitaire au quotidien, malgré cette longue liste de remerciements. Au regard de mon tempérament, j'ai souvent manqué d'interlocuteurs avec qui échanger sur mon travail, de personnes extérieures pour me stimuler. Ces éléments ont sans doute été un terreau fertile pour les nombreux doutes qui m'ont touchés pendant la thèse. Ainsi, tout en gardant un souvenir positif de cette thèse, j'ai aussi appris à connaître ce dont j'ai besoin dans un travail pour être stimulé.

Je remercie toutes les personnes hors du cadre de travail, avec qui j'ai partagé des moments pendant ces 3 années. Tous ces liens ont pour moi été essentiels afin de m'aider à couper, relativiser et décompresser. Et puis parce que pendant ces trois ans, il n'y avait pas que la thèse dans la vie.

Je remercie l''ensemble des potes de Rennes avec qui j'ai partagé des soirées, des marchés le samedi matin, des verres et cafés au St-Germain, des festivals et autres activités culturelles

(aussi). J'ai évidemment une pensée particulière pour mes colocataires du 14bis quai Emile Zola. Je vous ai côtoyé au quotidien, la semaine et le week-end, ce qui fait bien plus que mes collègues finalement ! On m'a parfois dit « je ne sais pas comment tu fais pour être en coloc, moi je ne pourrais pas ». Quand je pense à tout ce que m'a apporté ce mode de vie, je me dis l'exacte inverse. C'était l'assurance d'avoir autour de soit des amis sur qui compter, avec qui discuter, rigoler les soirs d'hivers en semaine, partager des activités… Cet entourage m'a fait chaud au cœur et m'a permis d'avoir une assise solide à côté de la thèse. On a même été plus loin dans le partage de nos vies, en partant ensemble en week-end bateau, en festivals ou encore à ce fameux rendez-vous annuel des Gras de Douarnenez. Bref, cette petite vie en communauté d'amis était un élément clé de ces de la réussite de ces trois années.

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Rennes, en octobre 2025, quelque part à proximité de la place St-Germain.

General introduction

Food is a necessary commodity for animals to sustain life. Food originates from other living organisms which can be plants, micro-organisms, other animals...etc. It is made of macronutrients (and micronutrients) which are ingested and broken down along their travel in the digestive tract. Resulting nutrients can then be absorbed by the animal and used by different organs and tissues. Nutrients can be used for various purposes such as building block such as energy sources or precursors for the synthesis of other molecules by cells of the organism. The process during which macronutrients are broken down and smaller nutrients are absorbed is called digestion. One important class of macronutrients are proteins, which are found in all types of living organisms and perform numerous functions, from signalling, structure, defence against pathogens, regulating gene expression…etc. Proteins are made of an assemblage of a hundred to several thousands of smaller nutrients, called amino acids (AA). After absorption these AA will then be re-used by the organism for the construction of new proteins, the synthesis of other molecules, as energy sources... Lack of certain AA in the diet over a prolonged period is known to impair essential biological functions and can lead to death. Protein and AA nutrition is thus essential and is in the spotlight of nutritionists for both animals and humans.

During digestion, the entirety of dietary AA transit and are absorbed through the small intestine (SI), making it a crossroad of whole-body amino acid metabolism. Thus, the metabolic use of AA by the SI has the potential to influence appearance of AA in the blood and their availability for post-absorptive metabolism. Metabolic activity is very high in the SI, as illustrated by daily replacement of intestinal proteins (Sève et al., 1993) or the oxygen consumption of the digestive tract, representing 20% of whole body oxygen utilisation (Yen et al., 1989). This metabolic activity is driven by the two main functions of the SI which are digestion and protection. On one side, the SI absorbs dietary nutrients liberated during the process of digestion, as well as secretes enzymes for the final hydrolysis of macronutrients. On the other, the SI protects the organism from the outside world, via the maintenance of a protective barrier supported by a secretion of mucus and the presence of numerous immune cells. All together it is the support of these two contrasting functions over a surface of 30 m^2 in adult humans (Helander and Fändriks, 2014) that drives an important demand for nutrients, including AA.

The pig, in addition of its agronomical interest, has been widely used as a model for human nutrition (Roura et al., 2016). Thus, it is probably one of the animal species for which digestion is best known and on which numerous *in vivo* nutritional studies have been performed. Several

authors have studied the influence of the SI on appearance of AA in the blood using the pig; they estimated that roughly 50 % of the dietary essential AA absorbed were not appearing in the blood, thus considered as metabolised by the SI (Bertolo et al., 1998; Stoll and Burrin, 2006). This result is intriguing, as the SI represents only 3-4 % of whole-body protein mass (Agyekum et al., 2012), it seems unexpected that it takes up to 50% of the dietary supply. The shared of dietary AA which are metabolised by the SI, thus not appearing in the blood is called firs-pass metabolism. Quantification of first-pass metabolism is highly variable among AA, with for example glutamine being entirely metabolised and alanine synthesised *de novo* by the SI. Quantification of intestinal metabolism of AA is complicated *in vivo* by the presence of two routes of nutrient supply, the length of the SI and rapidity at which metabolic processes occur (Leterme et al., 1996). In addition, *in vivo* experiments to study intestinal metabolism require invasive protocols which are to be limited in the coming years due to ethical concerns. Thereby, new methods are needed to be able to quantify the metabolic use of dietary AA by the SI during digestion.

In silico or mathematical modelling approaches have been widely used in animal science, mainly to study digestive processes (Bastianelli et al., 1996) but also for post-absorptive metabolism (van Milgen et al., 2008). The idea of modelling SI metabolism has been introduced by Bannink et al. (2006) and put in practice by Mills et al. (2017) with a focus on glucose metabolism by the SI in dairy cows. In human nutrition, modelling absorption of nutrients in the SI has also been pinpointed as a topic of interest for future research (Le Feunteun et al., 2021). Above mentioned difficulties encountered to measure intestinal metabolism of AA could be partly overcome by a modelling approach. Only a limited amount of data would be able to parametrize such model, yet the main biological processes and their order of magnitude are known.

In vitro approaches using cultured absorptive cells offer the possibility to have a reduced and controlled model of intestinal cell layer to perform variety of experiments. Cultured intestinal cells have been used to measure preferred energetic substrate of enterocytes (He and Wu, 2022) or the ability of enterocytes to degrade certain AA (Chen et al., 2009). The use of cultured intestinal cells to quantify the metabolic fate of AA is to our knowledge limited, with exception made of one recent study (Sakai et al., 2020). Several intestinal cell lines are now widely used and can be cultured in a polarised manner, to mimic the organisation of the intestinal mucosa (Hevia et al., 2023). These cell models offer a promising tool to study intestinal AA metabolism

in a controlled environment. In addition, they could be a solution to obtain data or elucidate mechanisms, which could in turn fuel a mathematical modelling approach.

Thereby, this thesis studied the intestinal metabolism of AA by combining an *in silico* and an *in vitro* approach. The first objective of this work was to better understand how intestinal metabolism influences the appearance of AA in the blood during digestion and interacts with digestive processes. Second, this thesis aimed to assess the interests of methods which are alternatives to animal experiments, to study digestive physiology and metabolism in animals.

The following literature review was done with the aim of gaining knowledge about intestinal metabolism of AA, in the view of choosing the appropriate modelling and cell culture approaches. The review begins with an overview of the structure of the SI (1.), followed by a description of protein digestion in the SI (2.). Then, metabolism of AA in the intestinal mucosa is described (3.) as well as methods to quantify AA fluxes in the SI *in vivo* in pig (4.). Finally, an overview of the interest of mathematical models in nutrition and animal science is given (5.) as well as on the use of *in vitro* cultured intestinal cells in nutritional studies (6.).

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List of abbreviations

AA: Amino acid

AID: Apparent ileal digestibility

ATP: Adenosine tri-phosphate

BCAA: Branched-chain amino acids

C: Carbon

DM: Dry matter

DMEM/F12: Dulbecco's Modified Eagle Medium F12

DMI: Dry matter intake

DTB: Dual tracer portal balance

EAA: Essential amino acids

IRMS: Isotope ratio mass spectrometer

FBS: Foetal bovine serum

FPB: Fractional portal balance

FSR: Fractional synthesis rate

GIT: Gastrointestinal tract

Gln: Glutamine

GSH: Glutathione

HBSS: Hans balance salt solution

HCl: Hydrochloride

LCA: Life cycle analysis

LY: Lucifer yellow-CH di-potassium

Lys: Lysine

N: Nitrogen

NEAA: Non-essential amino acids

NO: Nitric oxide

NPB: Net portal balance

O: Oxygen

P-5-C: pyrroline-5-carboxylate

PB: Portal balance

PDV: Portal drained viscera

PLF: Precision livestock farming

PS: Porcine serum

RPMI: Roswell Park Memorial Institute medium 1640

SI: Small intestine

SID: Standardized ileal digestibility

TEER: Trans-epithelial electrical resistance

TID: True ileal digestibility

Valorisations of the work conducted in this thesis

In a scientific journal

Garçon, C. J. J., Le Floc'h, N., Mercier, Y., & van Milgen, J. 2025. A conceptual mechanistic model of amino acid fluxes in the small intestine, taking the example of pig. Animal, 19(2), 101414.

Garçon, C. J. J., Le Floc'h, N., Mercier, Y., & van Milgen, J. 2025. Challenging a mathematical model of amino acids metabolism in the small intestine, developed in pigs. *Submitted to Animal.*

Communications in congress

Garçon C.J.J., van Milgen J., Le Floc'h N., Mercier Y. In silico approach of metabolic fluxes of amino acids in the small intestine with pig. International conference on food digestion, 9-11 April 2024, Porto, Portugal. (POSTER)

Garçon C.J.J., van Milgen J., Le Floc'h N., Mercier Y., 2023a. A modelling approach to investigate metabolic fluxes of amino acids in the small intestine of pigs. Book of abstracts of the 74th annual meeting of the European Federation of Animal Science, EAAP, 26 August – 1 September 2023, Lyon, France, p.474. (ORAL)

Garçon C.J.J., van Milgen J., Le Floc'h N., Mercier Y., 2023b. Modélisation des flux métaboliques d'acides aminés dans l'intestin grêle. 56èmes Journées de la Recherche Porcine, 6-7 February 2024, Saint-Malo, France, p.121–126. (ORAL)

Other communications

Garçon C.J.J., van Milgen J., Le Floc'h N., Mercier Y. Developing an *in vitro* method to study amino acid metabolism in the small intestine. Scientific days of the doctoral school EGAAL. 3rd of April 2025, Paimpont, France. (ORAL)

Garçon C.J.J., van Milgen J., Le Floc'h N., Mercier Y. The use of cultured cells to study amino acid fluxes in the small intestine. Webinars of the PIGWEB Junior Community. 16th of September 2024, online. (ORAL)

Chapter 1. Literature review

1. Structure of the small intestine

1.1. General features of the small intestine

In adult pig, the SI can reach 20 m long, rendering it the longest organ, starting from the stomach after the pylorus until the large intestine, at the ileocecal junction (McCance, 1974). In mammals, a long SI has often been associated with more herbivorous diets, while carnivorous were considered to have shorter SI, but this belief is now considered as false (Duque-Correa et al., 2021). In human, it is estimated that the whole surface area of the digestive tract is approximately 32 m^2, with 30 m^2 being SI surface (Helander and Fändriks, 2014): stressing the importance of the surface covered by this organ. It is usually divided in three distinct morphological segments, the duodenum (≈ 5 % of total length), the jejunum (≈ 35 %) and the ileum (≈ 60 %, Boudry et al., 2004). Although morphological differences exist in between these segments, it is important to bear in mind that there is no clear cut between them and that they share numerous common features. In human, the SI composed of 13 % proteins, 6 % lipids and 81 % water, which is relatively low in protein and high in water compared to other organs of the digestive tract. In comparison, skeletal muscle contains 75 % water and 20 % proteins (Woodard and White, 1986). Overall, the SI represents roughly 3 – 5 % of whole body mass in pig, depending on factors such as the age of the animal or diet composition (Adeola and King, 2006; Bolhuis et al., 2007).

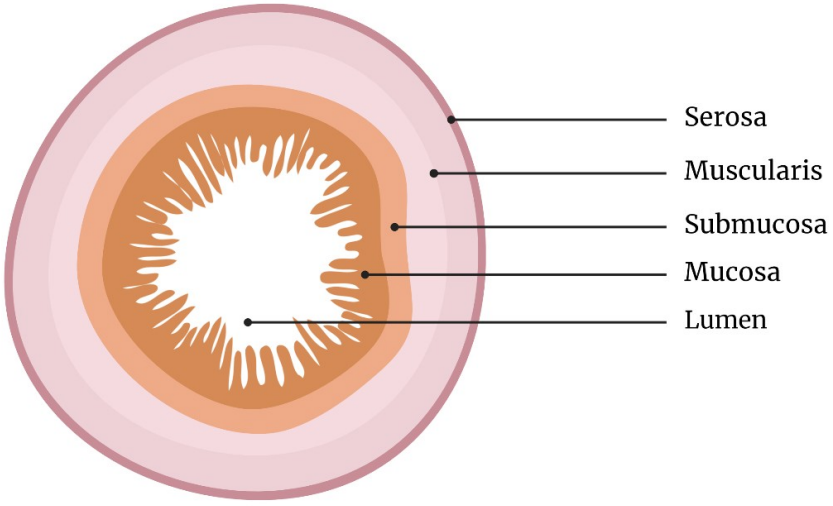


Figure 1. Scheme of a cross section of the small intestine.

1.2. Tissues and structural organisation

The SI is composed of four different layers of tissue which are the serosa, muscularis, submucosa and mucosa (**Figure 1**). The serosa is the outermost layer covering the SI and composed of a mesothelium, containing connective tissue, blood vessels and nerves. The muscularis layer is involved in intestinal motility and is composed two layers, a longitudinal outer layer and a circular inner layer. The submucosa is a layer of loose connective tissue, comprising blood and lymphatic vessels as well as nerves. The mucosal layer is composed of three different layers, the muscularis mucosa, the lamina propria and an epithelium (**Figure 2**). The lamina propria provides structural, vascular and neural support to the underlying epithelium (Mosenthin, 1998). The epithelium is lining the intestinal lumen and is in direct contact with luminal content. It is composed of a single columnar layer of cells, covered by a protective layer of mucus. The lining of the intestine, is made of finger like projections in the lumen called villi as well as folds called crypts. This uneven structure, made of villi and crypts, allows the SI to display a large surface area of exchange, as above mentioned.

1.3. Cell types composing the intestinal tissue

Most of epithelial cells originate from stem cells in the crypts, which migrates when differentiating until the tip of the villi (**Figure 2**). The epithelium is the layer of tissue comprising the most diverse cell types. Wiarda et al. (2023) denoted six cell types, represented all along the intestinal epithelium of pigs, in various proportions. The most represented cell type being the absorptive cells, namely enterocytes, which represent around 65 % of cells in human, followed by goblet cells (15%) and stem cells (10%) other cell types being minor (immune, Paneth or neuroendocrine cells). Numerous immune cells are also present in the intestinal mucosa, including lymphocytes, dendritic cells or M cells (McGhee and Fujihashi, 2012). Cell renewal in the intestinal epithelium is very high, lifespan of epithelial cells is estimated to range between 3 to 5 days (Arike et al., 2020; Sarri et al., 2024), dead cells being desquamated in the intestinal lumen.

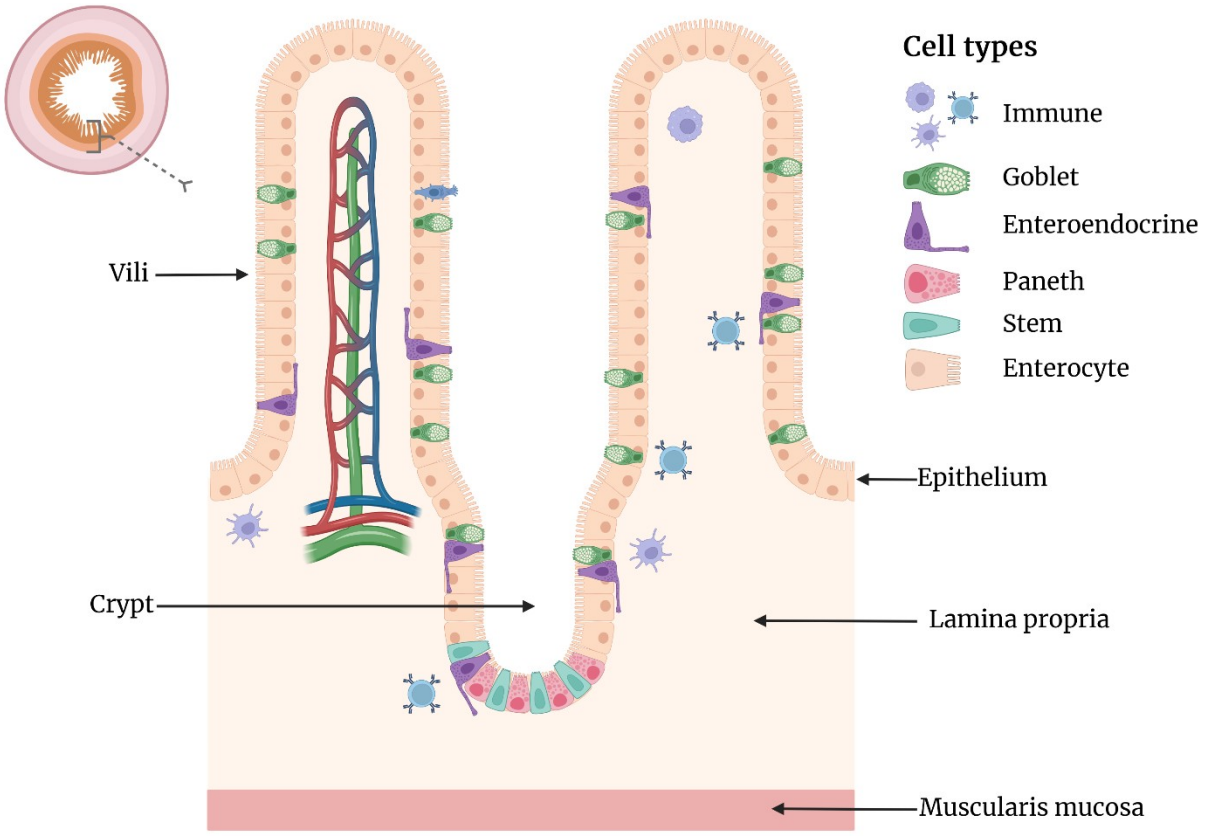


Figure 2. Scheme of the structure of the small intestinal mucosa and most represented cell types.

1.4. Conclusion on the structural features of the small intestine

The SI cannot be considered as a simple tube but has a complex three-dimensional structure and is composed of different layers of tissues and cell types. In the view of constructing a mathematical model, all these structural elements cannot be retained because they would require too much complexity. As an illustration, the tubular structure and the relative homogeneity along the SI are probably more important features to consider in a mathematical model than the morphological differences in between the three functional segments. Similarly, for cell culture, the presence of numerous cell types (including immune cells) or the three-dimensional structure of the epithelium are important features of the SI but complicated to replicate in a laboratory set-up. Focusing on the culture of enterocytes in a polarised manner, which are absorptive cells and the most abundant cell type seems to be a reasonable simplification. Both the *in silico* and *in vitro* approaches need to focus on elements of the intestinal structure which are relevant with the aim of investigating AA metabolism.

2. Protein digestion in the small intestine

2.1. Entrance of digesta and transit

The luminal environment is subject to the alternating meal pattern, thus periods of abundance in nutrients and intense activity are followed by periods of rest, without luminal provision of nutrients. The first element influencing the arrival or not of digesta in the SI is the feeding pattern of the animal. Animals with *ad libitum* access to feed, such as poultry or grazing cows, have an almost constant supply of nutrients to the intestinal lumen. Animals such as pig or human, often eat following a meal pattern; hence have to deal with a more periodic entrance of material in the lumen of their gastrointestinal tract (GIT).

After ingestion, arrival of digesta in the intestinal lumen is controlled by gastric emptying as reviewed by Liu et al. (2021). Gastric emptying is influenced by properties of the diet, such as volume, caloric content or viscosity (Gregory et al., 1990; Lannuzel et al., 2024). From an organisational point of view, stomach act as a batch in which digesta enters, is mixed, partially digested and stored, before a progressive emptying in a long thin tube which is the SI. In human, the first fraction of digesta being evacuated from the stomach are the liquids and soluble, which are emptied in 10 to 120 min depending on their caloric content (Goyal et al., 2019). Inert liquids are evacuated very quickly, at a constant rate resulting in an exponential curve while calorie rich liquids exhibit a linear emptying rate, due to gastric sieving (Liu et al., 2021). Solid particles are retained longer in the stomach, especially if they are of large size, to be broken down. It is generally assumed that mean retention time of solid particles in the stomach is around 2-3 hours, however, it was recently demonstrated that it could reach up to 10 hours for fibrous particles in pig (Lannuzel et al., 2024). These results together imply that digesta will enter progressively in the SI, divided in fractions according to their physicochemical properties, first the liquids, then solids and finally coarse particles of fibrous material.

As the SI is a long tube, digesta moves along and is in contact with only a fraction of the intestinal epithelium at the time. This movement along the SI is mediated by peristaltic contractions, which in addition to allowing plug flow, contribute to mixing and digestion (Lentle and Janssen, 2008). Retention time in the SI of growing pigs seems to lie in between 2 to 4 hours for both solid and liquid phase, and to be little influenced by dietary factors such as fibre content (Wilfart et al., 2007; Lannuzel et al., 2024).

2.2. Protein hydrolysis

The hydrolysis of dietary proteins (as well as endogenous) starts in the stomach with the action of pepsin, coupled with the denaturation of proteins via hydrochloric acid (HCl; **Figure 3**). This first gastric phase of protein digestion is determinant for the following steps of protein hydrolysis; it has been demonstrated *in vitro* that virtually bypassing gastric phase decrease intestinal trypsin mediated hydrolysis by 77 % (Rio et al., 2021). This first step of protein hydrolysis results in large polypeptides. The second phase of protein digestion starts after entrance of digesta in the duodenum, when the proteases rich pancreatic juice and bile are released, in the duodenum. At this point, pH of digesta coming from the stomach, which is generally low (≈ 2-4), increases to reach a value close to neutral (≈ 8), which will remain during the entire transit trough the SI (Wang et al., 2015). Although bile is not directly involved in protein hydrolysis, it has an important role in facilitating protein digestion, especially when they are in emulsions (Mackie and Macierzanka, 2010). Several types of proteases with different specificities in their targeted peptide bonds are secreted by the pancreas. Pancreatic proteases are secreted in an inactive form and need to be activated; this activation is done through a cascade reaction, starting with the activation of trypsin when reaching contact with enteropeptidases lining the intestinal epithelium, followed by the activation of other enzymes (chymotrypsin, elastase, carboxypeptidase) by trypsin (Bhutia and Ganapathy, 2018). This process of enzyme activation might be a source of delay in the onset of proteases activity; thus, in intestinal protein hydrolysis. It is commonly admitted that the end products of pancreatic proteases activity are peptides of 6-8 AA long, while free AA represent a small fraction. Final hydrolysis of these peptides takes place at the lining of the intestinal cells, in the unstirred water layer, by brush border enzymes which are located inside the membrane of epithelial cells. The main end product of hydrolysis by brush border enzymes are tri or di-peptides (80 %), while free AA represent the smallest fraction (20 %). Although being present along the whole SI, brush border enzymes have a higher activity in the ileum than jejunum (Das and Radhakrishnan, 1974; Raul et al., 1988), probably due to the fact the rate of appearance of small peptides in the lumen gradually increases along the length of the SI. Overall, hydrolysis of proteins is a process ongoing gradually while the digesta is moving along the SI.

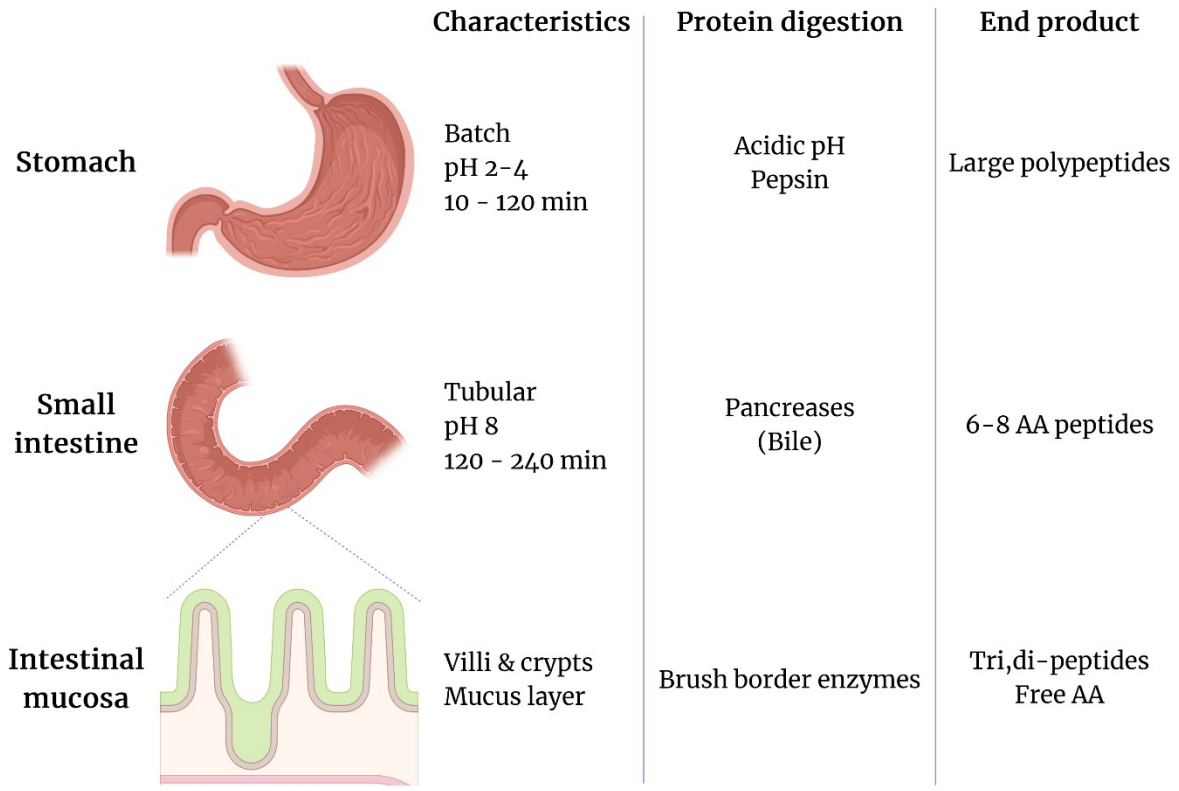


Figure 3. Summary of the main steps of protein digestion along the digestive tract.

2.3. Free amino acid and peptide transport across the intestinal epithelium

As mentioned above, AA absorption at the level of the intestinal mucosa is mediated by enterocytes which are intestinal absorptive cells. It is also admitted that most of AA absorbed from the lumen by the intestinal cells are transport as tri or di-peptides (80%) while free AA represent a small fraction (20 %, Bhutia and Ganapathy, 2018). Yet, to my knowledge there is no experimental study that confirmed this hypothesis. Transport of AA, in free AA or peptide form, can be classified according to the route taken (paracellular or transcellular) or the mode of transport (active transport, simple or facilitated diffusion). Via the transcellular route, transport can be mediated via active transport systems as well as passive, simple or facilitated diffusion. While through the paracellular route, passive diffusion is the only mode of transport possible.

Simple or facilitated diffusion are passive transport modes and can only follow the gradient of concentration while active transport systems can act against the concentration gradient via the use of co-transport of ions (Kakuda and MacLeod, 1994). Simple diffusion happens directly

through the lipid bilayer of the membrane of the cell while facilitated diffusion happens thanks to channel or carrier proteins located in the cell membrane (**Figure 4**). Active transporters present in the cell membrane are a classified as secondary active transporters and use an ion gradient created by a primary active transport system (mainly Na^+K^+ ATPase) to transport AA. Transcellular route also comprises transcytosis, which is the uptake of AA inside membrane vesicles. The importance of this mode of transport is less documented, although it is known to occur for peptides (Wang et al. 2019). The paracellular route of absorption is the diffusion of AA in between cells, in pores left by tight junctions which restrict passage of molecules in between cells (Stevenson and Keon, 1998). *Via* this route, AA transported do not enter the cytosol of enterocytes by directly reach the lamina propria (**Figure 4**).

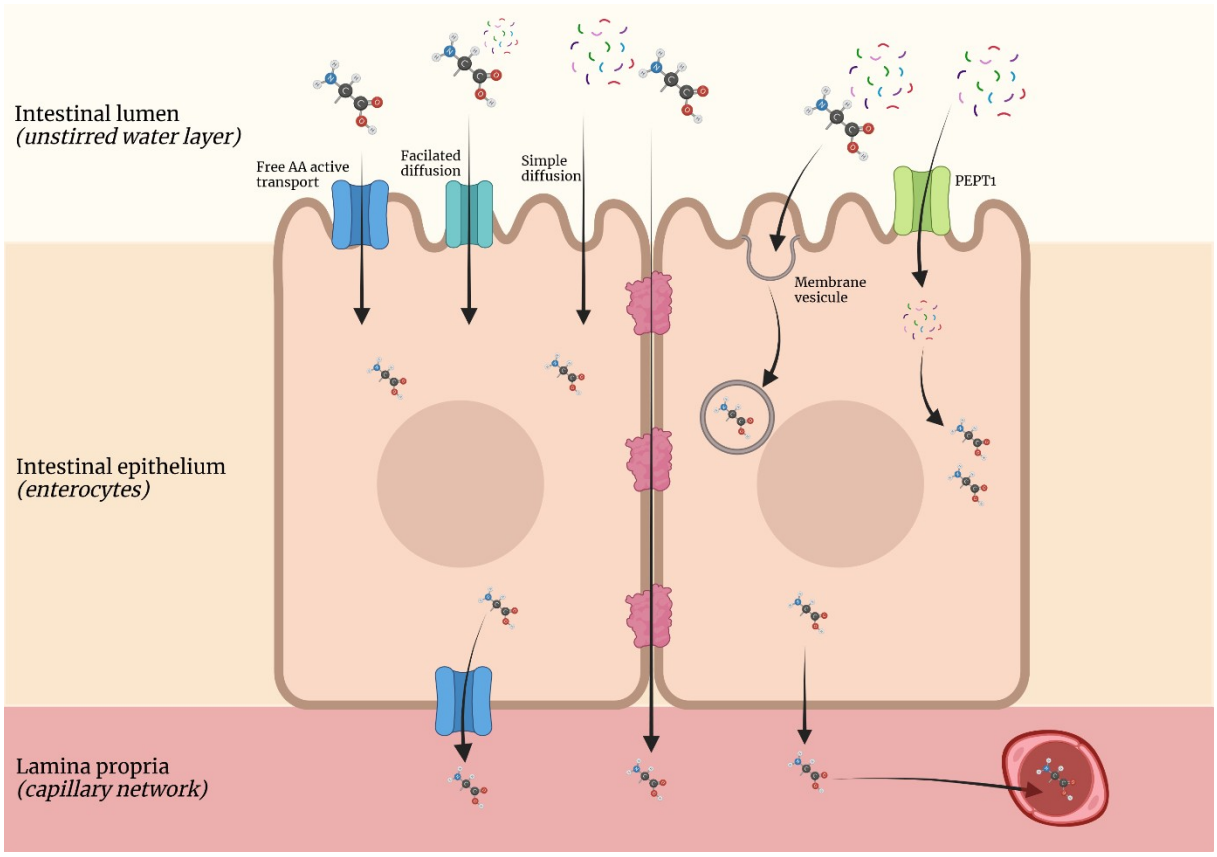


Figure 4. Scheme of amino acids transport systems available in the intestinal epithelium.

Secondary active transport in enterocytes is driven by transmembrane ion gradients as well as membrane potential (Bhutia and Ganapathy, 2018). At the root of active transport systems are the primary active transport system fuelled by ATP, Na^+ K^+ ATPase located in the basolateral side of enterocytes membranes. They pump three Na^+ out for two K^+ inside the cytosol, creating a gradient of Na^+ and K^+ in between the inside and the outside of the cells, and also generating a negative membrane potential. The NHE3 pump is a transporter of ions, pumping Na^+ inside the cell and H^+ outside, which generates an important H^+ concentration gradient in between the lumen and the cytosol. In addition, K^+ channels facilitate the efflux of K^+ from the cell at the basolateral side, re-enforcing the negative membrane potential, while Cl^- channels facilitate the efflux of Cl^- from the apical side.

Ions fluxes are at the basis of seven secondary active transport systems which are available for free AA absorption in enterocytes, as well as the unique PEPT1 transport system for peptides (**Table 1**). Only the $b^{0,+}$ system is dependent on fluxes of neutral AA as driving forces, instead of ions. Most of transport systems for free AA are Na^+ dependent (6). Each active transport system can transport several AA and most of them overlap in their substrate specificity, which can lead to situations of competition in between free AA for transport. Peptide transport, via PEPT1 is dependent on intra-luminal H^+ gradients, which is created by the NHE3 pump above mentioned.

Table 1. List of existing transport in the apical side of the intestinal brush border, with their driving force and specificity[1].

Transport system	Hugo nomenclature	Driving force	Location	Mechanism	AA transported
B^0	SLC6A19	Na^+	Apical	S[2]	Gly, Ala, Ile, Val, Leu, Phe, Pro, Met, Ser, Thr, Tyr, Cys, Gln, Asn, Trp
A	SLC28A2	Na+	Ubiquitous	S	Gly, Pro, Ala, Ser, Cys, Gln, Asp, His, Met
x^-_c	SLC3A2/SLC7A11		Ubiquitous	A	Glu
y^+	SLC7A1		Ubiquitous	U	Arg, Lys, His
y^{+L}	SLC3A2/SLC7A7	Na+	Basolateral	A	Arg, Lys, Gln, His, Met, Leu
$B^{0,+}$	SLC6A14	Na^+, Cl^-	Apical	S	Gly, Ala, Ile, Val, Leu, Phe, Pro, Met, Ser, Thr, Tyr, Cys, Gln, Asn, Trp, Arg, Lys, His
X^-_{AG}	SLC1A1	Na^+, K^+, H^+,	Apical	S	Asp, Glu
ASC	SLC1A5	Na^+	Apical	A	Ala, Ser, Cys, Thr, Gln
$b^{0,+}$	SLC7A9-SLC3A1	Neutral AA	Apical	A	Arg, Lys, His
IMINO	SLC6A20-ACE2	Na^+, Cl^-	Apical	S	Pro
N	SLC38A5	Na^+, H^+	Ubiquitous	S	Gln, Asp, His
PAT	SLC36A1	H^+		S	Gly, Ala, Pro
β	SLC6A6	Na^+, Cl^-	Ubiquitous	S	Taurine, β-Ala
PEPT1	SLC15A1	H+		S	di-tripeptides

[1]Adapted from (Bhutia and Ganapathy, 2018; Bröer and Fairweather, 2018).

[2]S, symport, U, uniport; A, antiport.

As reviewed by Webb (1990) the relative importance of each of these routes is dependent on luminal substrate concentration *in vitro*; suggesting that factors such as meal characteristics or location in the SI might influence the route of transport of AA. To our knowledge, there is no existing *in vivo* study quantifying the importance of each route of absorption for peptides or free AA in the SI during a meal. The fact that some chronic diseases are linked to a deficiency in the expression of certain transporters in the SI (Bröer and Fairweather, 2018) suggests that passive processes (paracellular or transcellular) are not sufficient alone. For peptides, there seems to be an inverse relationship between apparent permeability and molecular size, *in vitro* using rat intestinal epithelium (Dodoo et al., 2000). Another *in vitro* study demonstrated that other factors such as charges interactions or secondary structure of peptides can also influence their transport by intestinal cells (Wang et al., 2019). It can be speculated that paracellular diffusion gains importance in cases such as for new born or during intestinal inflammation, when intestinal permeability is increased (Lallès et al., 2004; Hu et al., 2013). Buddington et al. (2001) studied the relative contribution of diffusion to total absorption of AA with isolated cells sampled in pig at different ages. Contribution of diffusion to absorption was estimated to be around 20-30 % for free Leu, Lys, Met and Pro. This seem to indicate a significant contribution of diffusion to absorptive process; however, whether it is the case *in vivo* remains questionable. In fact, in the experiment of Buddington et al. (2001) *ex vivo* intestinal tissue was used, which integrity could have been impaired by the protocol. In the same study, after weaning, an increase by 49% in the contribution of diffusion to absorption of AA was observed compared to before weaning; probably due to the alteration in intestinal integrity induced by weaning (Lallès et al., 2004). In summary, it seems that during digestion, active transport is the most important route for AA transport, although passive transport probably plays a quantitatively significant role, especially in situation when integrity of intestinal mucosa is affected.

Several authors highlighted the regional variation in the expression of transporters along the SI in pig and mice (Mutch et al., 2004; Wang et al., 2009); thus in the transport capacity of AA. In their review, Bhutia and Ganapathy (2018) stated that absorptive capacities for peptides are greater in the proximal than distal SI, whereas it would be the opposite for free AA. This could be linked to the pattern of free AA and peptides release, as the proportion of free AA generated by protein hydrolysis might increase along the SI, while the quantity of peptides decreases.

Finally, there seems to be interactions between diet composition and the expression of AA transporters along the SI. It is known that both the form of arrival of AA in the lumen, as intact protein or free AA (Eugenio et al., 2023) and the quantity and profile of AA in the diet (García-Villalobos et al., 2012) influences expression of AA transporters in the SI of pigs.

2.4. Endogenous protein fluxes

The concept of endogenous fluxes encompass fluxes of proteins which originate from the organism itself and ends in the lumen of the GIT, with different origins in term of site and nature. They can be cells, mucus, gastric juice or enzymes (salivary amylase) from the upper digestive tract mixed with digesta in the stomach and passing through pylorus. It can also be bile or pancreatic juice, entering in the duodenum at the site of the major duodenal papilla, in the duodenum. Finally, these endogenous fluxes can be from intestinal origin, being desquamated intestinal cells, mucus or brush border enzymes. Endogenous fluxes from non-intestinal origin enter in the upper SI, while fluxes from the intestine itself can originate from the whole length of the SI. It is difficult to estimate the relative contribution of each of these organs (stomach, pancreas, bile, SI) or forms (mucus, cells, enzymes…) to the total amount of secretions. *In vivo* studies allow to measure ileal losses rather than secretions *per se*. In a study cited by Nyachoti et al. (1997), Low et al., 1985 estimated that 60 % of nitrogen (N) endogenous losses were from intestinal origin. Nevertheless, the method used for this estimation is unknown and the value must be taken with care. Because intestinal secretions are the most distal ones as mentioned above, it is likely that they are the less well reabsorbed; thus end up as the main losses. In literature, these fluxes of endogenous nutrients, especially proteins are often termed endogenous secretions (Leterme et al., 1996), yet they do not all imply a mechanism of secretions *per se* – as illustrated with desquamated cells.

2.5. Protective functions

While the SI is a large surface of exchange, permissive enough to allow the absorption of nutrients, it also potential route of entry for pathogens. During digestion, the SI plays a protective role against the harmful external environment and potential pathogens. A layer of mucus is secreted along the epithelium with a thickness of 26 to 31 µm, increasing along the SI (Varum et al., 2010). This mucus layer is eroded during digestion, especially with fibre rich diets and has to be renewed (Montagne et al., 2003). It seems that the increase in mucus

production due to inclusion of dietary fibre in the diet compared to a control diet can be up to 200% (Montagne et al., 2003). In addition, antibodies, especially IgA are secreted in the intestinal lumen and can represent 3-5 g/d in human (Conley and Delacroix, 1987). Other immune related peptides, such as defensins are secreted in the intestinal mucosa (McGhee and Fujihashi, 2012). All these peptides secreted by the immune system might drive an important demand for AA, especially in situations of immune challenge. The SI is also the site of numerous interactions in between the immune system and microflora (Cerf-Bensussan and Gaboriau-Routhiau, 2010), but these aspects are beyond the scope of the present work.

2.6. Efflux of AA at the basolateral side of enterocytes

The efflux of AA from the basolateral side of enterocytes after absorption is an area under studied compared to the apical absorption. Several active transporters are expressed at the basolateral side, often being shared with the apical membrane (**Table 1**). They allow the transport of free AA in the extracellular medium, against the ion gradient. The relative importance of passive diffusion to active transport across the basolateral membrane is to our knowledge, not elucidated yet. It can be speculated that the gradient of concentration of AA in between the extracellular medium and the cytosol of enterocytes influences the relative importance of passive diffusion at the basolateral side compared to active transport. Thus, the rate at which AA are taken from the extracellular medium to the capillary networks and the portal vein has probably an influence on the relative importance of these mode of transport.

Most of AA appearing in the blood are in the form free AA, while peptides are the major form of absorption as reviewed by Bhutia and Ganapathy, (2018). Low level of tri-peptides are found in the blood after digestion (<0.1 % of intake), even when directly infused intra-gastrically (Have et al., 2015); suggesting than only a tiny fraction of dietary tri,di-peptides reaches the blood.

2.7. Conclusion on protein digestion in the small intestine

Processes of entrance of digesta in the SI and subsequent protein digestion are complex, involving numerous enzymes with different specificities. Such process could require a dedicated modelling approach and is beyond the scope of this thesis. In the perspective of this thesis, it can be retained that hydrolysis is gradually occurring through enzymatic activity, while digesta is transiting along the SI. Similarly, transport of AA at the apical side of intestinal

epithelial cells is very complex, and the relative importance of the different pathways not fully elucidated yet. However, it seems that it occurs mainly via active transporters, which are not specific for one given AA. With regard to the cell culture, processes of protein digestion are beyond the scope of our experimental approach. However, an *in vitro* set-up could allow to study transport of AA at the level of the apical membrane as well as basolateral efflux of AA.

3. Metabolism of amino acids by intestinal cells

3.1. Peptide hydrolysis

After being absorbed by enterocytes, AA can be used for various purposes such as protein synthesis, energy furniture, *de novo* synthesis of other molecules or AA. To this end, free AA can be directly used, while di or tri-peptides need to be broken down in free AA. Presence of di-peptidases has been confirmed in the cytosol of enterocytes from pigs (Norén et al., 1977). In guinea pigs, it has been shown that while di-peptidases are mainly present in the cytosol (>80% of recovered activity), tri-peptidases, are also present in the brush border (≈ 50% of recovered activity, Peters, 1970). There seems to be a species effect in between rat and guinea pig on di-peptidases localisation, while it is not the case for tri-peptidases (Wells et al., 1979). To sum up, it seems that although there might be differences in cellular repartition of tri and di-peptidases in enterocytes, they are both present and active in the cytosol. The activity of these enzymes in the cytosol of enterocytes explains why only a small fraction of tri,di-peptides are found in the blood during digestion (Have et al., 2015), as earlier mentioned.

3.2. Amino acid catabolism

2.1.1. Glutamine, glutamate, aspartate

In nutrition, AA are usually divided in two categories – essential (EAA) and non-essential (NEAA) amino acids – based on the ability of the organism to synthesise them *de novo*. Several studies in pigs, rats and humans showed that the SI uses NEAA as main energy source instead of glucose (Wu 1998). The pioneering studies of Windmueller and Spaeth (1980) showed that the SI of rats metabolises most of the dietary glutamate, glutamine, aspartate and arginine for ATP synthesis. In piglets, it was found that half of the enteral glutamate was converted into CO_2, which is quantitatively more than the use of systemic glucose (Stoll et al. 1999); suggesting that glutamate is the main energy source for the intestinal mucosa. *In vitro*,

enterocytes of new-born piglets used glutamine as main energy source, while those of suckling piglets produced more ATP from glucose than from glutamine (Wu et al. 1995). This result suggests that at least in the first days of life, glutamine is a more important energy source for intestinal cells than glucose. This result was confirmed in a subsequent *in vitro* study, which showed a higher rate of utilisation for glutamate, glutamine (and aspartate) over glucose with enterocytes from 0 to 21 days old piglets (He and Wu, 2022). It seems that both glutamate and glutamine are oxidised at similar extents by pig's enterocytes (Blachier et al. 1999), although glutamine needs to be converted into glutamate before being used (**Figure 5**). However, some results also suggested a higher rate of glutamine utilisation over glutamate in isolated enterocytes from piglet (He and Wu, 2022).

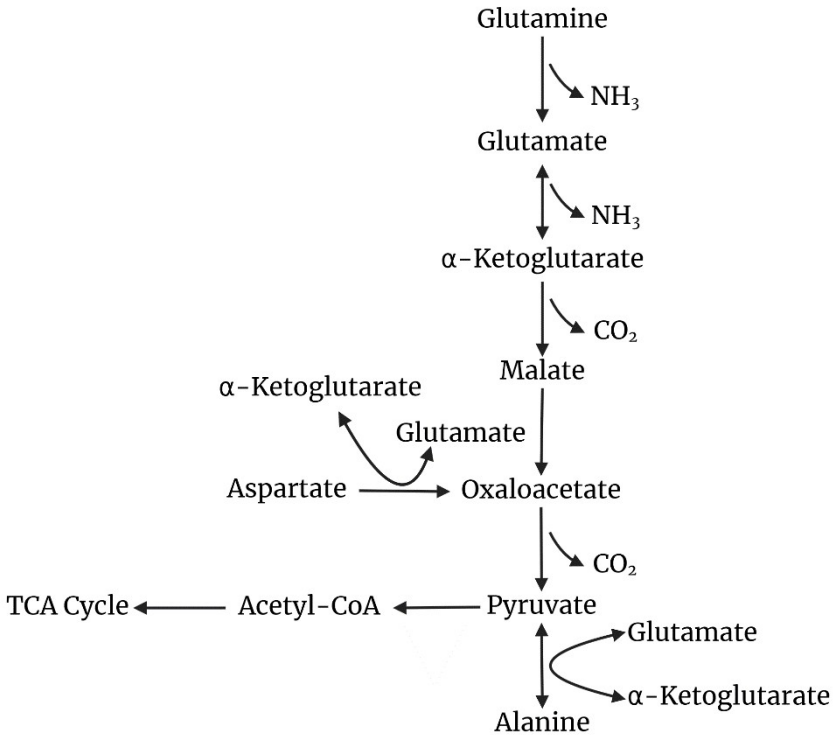


Figure 5. Metabolic pathways associated with glutamine, glutamate and aspartate catabolism in enterocytes.

In rats, it has been shown *in vivo* that aspartate was intensely metabolised by intestinal cells and that half of metabolised aspartate was used as an energy source (Windmueller et al. 1976). *In vitro*, it was also demonstrated that the rate of utilisation of aspartate was higher than that of

glucose with isolated enterocytes from 0 to 21 days old piglets (He and Wu, 2022). To our knowledge, the metabolic fate of aspartate in pig's enterocytes has not been studied yet. Deamination of aspartate would lead to production of oxaloacetate (and glutamate), which can then enter the Krebs cycle via pyruvate and acetyl-coa for a complete oxidation (**Figure 5**). Regarding arginine, only a very small fraction is oxidised to CO_2 in piglets (Wu et al. 1996), contrary to what was found in the small intestine of rats (Windmueller et al. 1976). Therefore, it seems that arginine is not an important energy source for pig's intestinal cells.

The catabolism of EAA by the cells of the mucosa could be regarded as a source of inefficiency, because it signifies that these EAA are "lost" for the organism. It has been shown *in vitro* that pig jejunal and piglet mucosal cells actively transaminate branched-chain amino acids (BCAA), but that resulting branched chain keto-acids are only oxidised to a small extent (Chen et al. 2007; Chen et al. 2009). Thus, it can be considered that BCAA are not a significant energy source for enterocytes. This active transamination of BCAA has been proposed to be a way to modulate the amount of BCAA entering blood circulation (Chen et al. 2009). With that regard, it is possible that in a situation of excess luminal BCAA, the transamination of dietary BCAA would increase to modulate their entrance in the systemic circulation. The catabolism of other EAA seems to be limited, because intestinal cells lacks the enzymes required for such purpose (Chen et al. 2007; Chen et al. 2009).

3.3. *De novo* synthesis of amino acids

3.1.1. Alanine synthesis

Alanine is an important product of catabolism of glutamine, glutamate, aspartate as well as potentially BCAA for the transport of nitrogen (Wu, 1998). Yet, level of ammonia in the portal blood after digestion is also high (Stoll et al., 1998), suggesting that a large shared of nitrogen from AA catabolism is also directly excreted in the form of ammonia. This nitrogen could also be converted into urea, as the ability of enterocytes to synthesise urea has been demonstrated in piglets (Wu, 1995). One study showed *in vitro* that a substantial amount of alanine is produced by enterocytes from suckling piglets incubated with glutamine in a buffer medium (Wu et al. 1995). Besides accepting nitrogen from the deamination of AA, alanine could also receive the carbon chain from the partial catabolism of glutamine, glutamate and aspartate (**Figure 5**). In this case, glutamine and glutamate would enter the Krebs cycle at the level of α-ketoglutarate

and leave the mitochondria in the form of malate, after losing two carbons in the form of CO_2. In this situation, the three remaining carbons would then be used to synthesise pyruvate, which can be transaminated to synthesise alanine.

3.1.2. Synthesis of other amino acids from glutamate and glutamine

As illustrated in **Figure 6**, dietary glutamate can also be used by intestinal cells for proline and arginine synthesis, both with pyrroline-5-carboxylate (P-5-C) as common precursor (Wu 1998). The enzyme involved in the conversion of glutamate to P-5-C are almost exclusively located in the intestine in adult pig (Wakabayashi 1983); implying that the intestine plays a major role in arginine and proline synthesis in the adult pig.

It has been shown *in vitro* with intestinal mucosal cells that synthesis of proline from glutamine is absent in suckling pigs, but present in weaned pigs (Flynn et al. 1997). The production of proline by the intestinal mucosa of weaned pigs, but not suckling piglets was also confirmed *in vivo* (Wu et al. 1994). The absence or presence of proline synthesis could be a dietary or developmental response. Besides, one study in weaned pig showed the SI to be the major site of proline synthesis from glutamate at the scale of the animal (Murphy et al. 1996). Therefore, it can be considered that proline synthesis might be an important fate for dietary glutamate and glutamine in post weaning and adult pig.

Arginine can also be synthesised from glutamate, via the carbamoyl phosphate pathway, which has two main intermediates – ornithine and citrulline (Salway, 2016; **Figure 6**). Ornithine and citrulline synthesis from glutamine is present in suckling pig and increased after weaning in enterocytes cultivated *in vitro* (Flynn et al. 1997). This increase seems to be due to the increased activity of the enzyme P-5-C synthase after weaning, which is involved in the synthesis of an intermediate of arginine and proline, from glutamine. Similarly, *in vivo*, the jejunum of suckling pigs produces citrulline, but at a much lower rate than post weaning (Wu et al. 1994). It was found *in vitro* that the production of citrulline and arginine by intestinal cells is important during the very first days post-birth, and that it becomes almost absent after 14 days of life in suckling piglets (Wu et al. 1995). These results were confirmed by another study with enterocytes cultured *in vitro*, which showed that arginine production from glutamate is present at birth, but not in suckling or post weaning piglets (Blachier et al. 1993). Arginine synthesis from ornithine and citrulline was found to be present at similar rates at birth and in suckling pig, but almost

absent post-weaning. It seems that the synthesis of arginine is mainly possible in enterocytes at birth, when the kidneys are not fully able to produce it. Later, this ability of arginine synthesis disappears, and enterocytes produce only its precursors, namely ornithine and citrulline. In fact, in adult pig, most of citrulline produced in enterocytes is released in the portal blood and converted to arginine in the kidneys (Flynn et al. 1996).

Arginine and proline can be converted to each other in the cytosol of intestinal cells (Wu 1998; **Figure 6**). It was found *in vitro* with intestinal mucosal cells that the synthesis of proline from arginine is negligible in suckling pig, while it is a major pathway for arginine degradation in weaned piglets (Wu et al. 1996). Contrarily, the reverse pathway from proline to arginine has been demonstrated *in vitro* to be fully functional in the enterocytes of both suckling and weaned piglets, although less important in the latter (Wu, 1997). One *in vivo* study in suckling piglets showed that proline was not an effective precursor for arginine synthesis, contrarily to citrulline, to compensate for an arginine deficient diet (Urschel et al., 2006). This result seems a bit contradictory with the outcome of Wu (1997) mentioned above, but presence of a metabolic pathway *in vitro* does not imply that it is sufficient to contribute metabolic processes as the whole body scale.

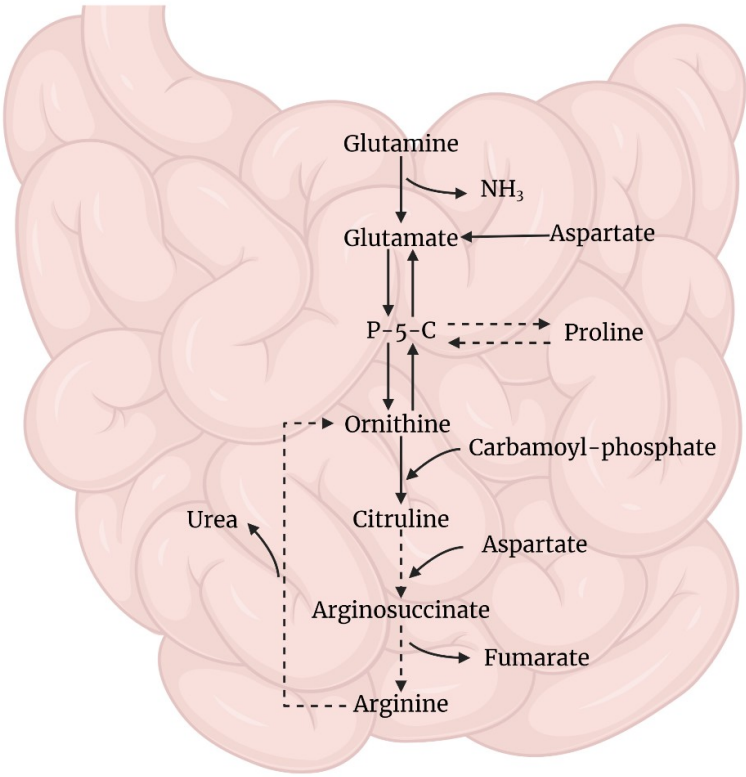


Figure 6. Arginine and proline related metabolic pathways in the small intestine. Dashed lines indicate processes which importance varies with physiological status. Adapted from Wu (1997).

3.1.3. De novo synthesis from essential amino acids

Essential amino acids seem not to be degraded significantly by intestinal cells (Chen et al. 2009), suggesting that they are not major precursors for the synthesis of any NEAA in the intestine. However, it should be highlighted that methionine, with serine, can be used as a precursor for cysteine synthesis (**Figure 7**). Speculatively, it could be a quantitatively important pathway, as cysteine is abundant in other metabolites produced by the SI such as mucins and glutathione (Mastrototaro et al., 2016). In piglets, it has been demonstrated that the intestine uses 12% of the dietary methionine intake for homocysteine synthesis (Riedijk et al. 2007). Homocysteine is an intermediate of cysteine synthesis, therefore it could be postulated that this pathway is present in intestinal cells and quantitatively significant. However, homocysteine can also be methylated back to methionine thanks to a methyl donor; thus not resulting in cysteine synthesis (**Figure 7**).

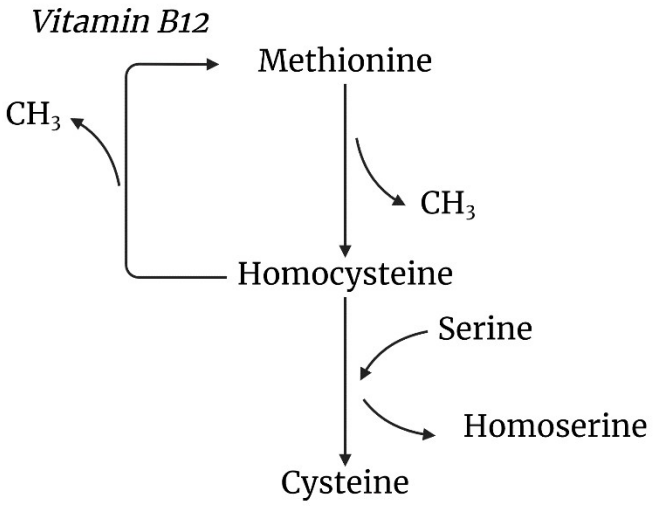


Figure 7. Metabolic pathway of cysteine synthesis from methionine, including the Salvage Pathway for methylation back of cysteine to methionine.

3.4. Protein synthesis metabolism

Intestinal mucosal cells synthesise proteins, whether it is for secretion, their own metabolic activity or cell integrity. The rate of protein synthesis in the intestinal mucosa is high and constantly drives the need for new AA to be taken up (Xi et al., 2012). The gastrointestinal tract and the liver together represent only 8 – 14% of body protein mass in monogastric animals, but account for 25 – 45% of whole-body protein synthesis (Lobley, 2003). Estimations of fractional synthesis rate (FSR) for intestinal proteins ranges from 50 to 120 % per day in pig as reviewed by Sarri et al. (2024), while it is estimated around 10 % per day in muscle tissue. This high rate of protein synthesis certainly leads to an important demand for AA, for example, in piglet 73% of methionine used by the SI was estimated to be directed to protein synthesis (Riedijk et al. 2007). A faster turnover was found for proteins related to energy metabolism (glycolysis and citric acid cycle), while it was slower for structural proteins (McConnell et al., 2011).

The synthesis of secreted proteins, such as mucins is an important driver of intestinal use of AA. Fractional synthesis rate of mucoproteins in rats was estimated to be 138 % per day in the ileum (Faure et al., 2002); which means that more than the entire mucus layer is replaced every day. This value was determined in the absence of a solid meal, which could stimulate an even faster replacement due to its abrasive effect of digesta on the mucosal mucus layer (Montagne

et al., 2004). As reviewed by Montagne et al. (2003), the amount of mucus recovered at the end of ileum increases up to 3 fold when fibrous material is added to a diet. However, it must be noted from the same review that in some studies, no stimulatory effect by fibre on mucin recovery in ileum was found; highlighting differences probably linked to physico-chemical properties of fibres. Total mucin output in ileal digesta was estimated to be around 5.5 g/day, in pig (Lien et al., 1997), although it could also contain mucin from the whole upper digestive tract. It must be noted that measurement of recovery of mucins at the end of ileum hides processes from secretions of mucus in the lumen, its detachment, hydrolysis and potential re-absorption or resulting AA, which can all be differentially affected by the presence of fibre in the lumen.

The most abundant of all AA in mucins is the EAA threonine, representing 16% of AA in mucins; together with proline and serine, they represent around 40% of total AA in mucins (Lien et al., 1997). Goblet cells which produce mucins are likely to have high requirements in these specific AA. Besides, as the mucus layer increases in thickness from the duodenum to the ileum (Varum et al. 2010), this might result in higher requirements in AA (especially threonine) for mucin synthesis in the distal parts of the SI. Schaart et al. (2005) showed that in a situation of protein restriction, most of the threonine utilised by the SI is of luminal origin; while it is more balanced with systemic intakes in case of normal protein supply. Conversely, Law et al. (2007) showed in piglets that parenteral threonine supply is efficient to compensate an oral deficiency of threonine regarding mucus production. From these results, it can be hypothesised that the intestine is able to adapt the origin of its threonine uptake, depending on where it is available. In minipigs, no increase in first-pass metabolism of threonine by the SI was found after ileitis infection, while the increase in total threonine uptake by the portal drained viscera (PDV) was 5-fold (Rémond et al., 2009). It is possible that in a situation of increased threonine demand, arterial blood supply becomes dominant for goblet cells. Another important aspect regarding mucins is that they are expected to be resistant to intestinal proteolysis, hampering their recycling (van der Schoor et al. 2002). This low recycling of mucins could represent an important loss of constitutive AA and especially threonine for the body.

Cells from the intestinal mucosa secret antibodies and antimicrobial peptides as defence mechanism against bacteria. The main antimicrobial peptide secreted by Paneth cells are α-defensins, which are typically 18-45 AA long contain a high proportion of cysteine (Lehrer and

Ganz, 2002). Their synthesis could be an important driver for cysteine uptake by the intestinal tissue. A large quantity of IgA is secreted in the intestinal lumen by plasma cells, level of secretion being influence by interactions with gut microbiota (Santaolalla et al., 2011). It was estimated with minipigs that 30 % of IgA present in the whole-body plasma originates from the intestinal mucosal synthesis (Vaerman et al. 1997). Thus, the intestinal mucosa is one of the main sites for IgA secretion in the body. These IgA being made of peptides, there secretion might lead to a certain demand in AA. Finally, peptides in antibodies are linked via sulphur bond; thus, it could be postulated that IgA synthesis also drives the demand for the catabolism of sulphur AA as sulphur donors. Immune related metabolic activity probably gains importance in situation of immune challenge and interacts with intestinal microflora.

3.5. Relations cellular energy metabolism

Consumption of energy by the PDV can be estimated by measuring the O_2 consumption. Dioxygen consumption by the PDV includes small intestinal O_2 consumption, among other digestive organs. Using results from Yen et al. (1989) and considering that one mol of O_2 consumed equals five mols of ATP produced, it can be estimated that ATP consumption by the SI of a 40 kg pig was 0.85 mol/hour over a 12 hour period in absence of a meal and 1.24 mol/hour with a meal. Using data from Yen and Nienaber (1993) who performed a kinetic over 6 hours post-prandial leads to a similar estimate around 1 mol/hour. It is not possible *in vivo* to go further and distinguish SI O_2 consumption from the rest of the PDV, yet these results provide an estimate of what maximum values could be attributed to the SI.

Some other studies have estimated *in vitro* the rate of O_2 consumption using *ex vivo* sampled intestinal tissue (**Table 2**). These results are in the range of those obtained for the whole PDV as mentioned above. However, it must be noted that *ex vivo* measurements cannot reflect the effect of alternating meals on energy consumption by intestinal cells. Results from these studies together also highlighted the fact that Na^+K^+-ATPase activity represented a significant shared of energy expenditures by intestinal cells (20 – 60 %) as reviewed by Cant et al. (1996). This would suggest that transport of nutrients via active transporters (and osmotic regulation) is one of the main drivers of energy consumption by intestinal cells.

We above mentioned the fact that NEAA – mainly glutamine, glutamate and aspartate – are intensely catabolised by intestinal as energy sources (Wu, 1998). Recently, one *in vitro* study

suggested that glutamate and glutamine are preferred to glucose as energy substrates (He and Wu, 2022). However, this was done with by measuring CO_2 production from with fixed concentrations of each substrate. *In vivo*, the actual importance of each substrate might also depend on their availability in the lumen or blood. The use of glutamine by intestinal cell implies the same metabolic pathways as glutamate, with the addition of a step of deamination (**Figure 5**). This leads to the production of NH_3 in the cytosol which would then need to be removed via the urea cycle, which has an energetic cost. If glutamine and glutamate are completely catabolised, one mol of these AA can produce 22 mol of ATP, without accounting for the energetic cost of NH_3 disposal (van Milgen, 2024). The above-mentioned partial catabolism of one mol of glutamate for one mol of alanine leads to the production of 7.25 mols of ATP. Catabolism of aspartate, when complete, leads to the production of 14.75 mol of ATP.

Table 2. Estimated ATP consumption from *ex vivo* sampled tissue in different animal species.

Author	Animal	Tissue	ATP consumption (mol/hour)[1]
Spratt et al., 1990	Chicken	Ileum	2
Summers et al., 1991	Chicken	Jejuno-ileum	4
Park et al., 1993	Chicken	Jejunum	4
Park et al., 1993	Chicken	Ileum	2
Bird et al., 1994	Mice	Jejunum	3
Pine et al., 1994	Rat	Duodenum	1
McBride and Milligan, 1985	Sheep	Duodenum	1
Kelly et al., 1993	Sheep	Duodenum	1
McBride and Milligan, 1984	Cow	Duodenum	1
Early et al., 1990	Cow	Jejunum	2
Kelly et al., 1992	Cow	Jejunum	2
Nyachoti et al., 2000	Pig	Jejunal mucosa	0.3

[1]Calculated from the initial value of O_2 consumption by the tissue as measured *ex vivo* (often in nmol/min/mg), considering a mass of SI of 857 grammes (Yen et al., 1989) and 5 mol of ATP produced per mol of O_2 consumed. Part of the data used for the calculation were obtained from (Cant et al., 1996)

3.6. Synthesis of amino acids derived molecules

Amino acids can serve as precursors for the synthesis of several metabolites, which are of high importance for maintaining SI functions as reviewed by Wu (2013). Glutathione (GSH) is a tri-peptide synthesised by intestinal cells among others, formed of glutamate, glycine and cysteine, which serves as a major antioxidant (Sies, 1999). The intestine preferentially uses enteral glutamate to synthesise GSH (Reeds et al. 1997). Thus, first-pass use of cysteine and glutamate might be closely linked to GSH synthesis by intestinal mucosal cells. In situation of

inflammation, when GSH synthesis is increased (Rakhshandeh et al., 2019), intestinal cells might need more cysteine to sustain GSH synthesis.

Nitric oxide (NO) is a nitrogenous compound known for its protective role in the intestine (Lanas, 2008). Arginine catabolism is limited in epithelial cells of piglets but increase after weaning, notably driven by the NO synthase pathway (Wu et al., 1996). The activity of NO synthase has been shown *in vitro* to be very low in the suckling pig and dramatically increased post-weaning (M'Rabet-Touil et al., 1993). Although arginine catabolism is quantitatively low, NO synthesis is likely to be an important fate of metabolised dietary arginine in adult pig (Wu et al. 1996). In the gut, urea can be synthesised from ammonia, glutamine and arginine in weaned pigs while it is not the case for suckling pigs (Wu et al., 1995). Speculatively, urea could be an important mechanism for the disposal of NH_3 issued from AA catabolism by intestinal cells. Urea production by intestinal cells could also be a co-product of proline synthesis from arginine.

3.7. Conclusions on amino acid metabolism in intestinal cells

This part summarised the numerous metabolic pathways implying AA identified in intestinal cells. Each of the proteogenic AA have their own specificities and can contribute to different processes in intestinal cells (e.g., methionine for GSH synthesis, glutamate to synthesise alanine…etc.). This diversity in metabolic pathways available cannot be considered in one single model as it would require too much complexity. However, a focus on elements which are common to each AA (protein synthesis, transport…etc.) would be relevant for constructing a model. Similarly, in a cell culture experiment, not all AA can be studied at the same time. However, some contrasting cases could be studied, for example NEAA which are used as energetic substrates (e.g., glutamine, glutamate, asparatate) and EAA which are only metabolised for protein synthesis. In fact, a relevant *in vitro* cultured cell model should metabolise differently these AA.

4. Assessment of amino acid fluxes across the small intestine *in vivo*

4.1. Concept of first-pass metabolism

The first use of the term first-pass metabolism or extraction dates from the early 1970s, from studies on absorption of drugs (Perrier and Gibaldi, 1972; Cohen et al., 1974). It seems that the initial concept of "first-pass" originated from the fact a fraction of an orally administered drug (acetylsalicylic acid) partly disappeared after its first passage through the gut wall and liver, before reaching systemic circulation (Rowland et al., 1967). Thus, the concept of first-pass referred to the phenomenon of extraction of a molecule by tissues (or organs) before reaching a defined target. In pharmacology, first-pass extraction occurs when a drug is metabolised between its site of administration and its site of action (Pond and Tozer, 1984). Often, studies in pharmacology include gut wall and liver metabolism when considering first-pass extraction of orally administered drugs (Zgair et al., 2020). Both first-pass metabolism or extraction are used in studies, designating the same processes, in the present document, we will use first-pass metabolism.

In nutrition, first-pass metabolism of AA was first studied in the late 1990s, one of the most cited study on this topic being from Stoll et al. (1998). The concept of first-pass metabolism was either used for the intestinal metabolism only (Reeds et al., 1996) or considering the whole splanchnic metabolism (Volpi et al., 1999). Later, studies on first-pass metabolism of AA have mostly focused on intestinal first-pass metabolism. This is likely due to the suspected high importance of intestinal activity on AA metabolism as reviewed by Wu (1998). In nutrition, most of the studies on AA first-pass metabolism have been published in between 1995 and 2010 and used catheterised pigs as model. In this document when we refer to first-pass metabolism, it corresponds the metabolic use of absorbed dietary AA by the small intestine, regardless of the purpose.

4.2. Methodologies to estimate first-pass metabolism

Intestinal first-pass metabolism of AA is a concept which is difficult to estimate *in vivo*. To our knowledge, three methodologies have been used to quantify first-pass metabolism, all based on the use of catheterised pigs. These animals have the advantage of being of a suitable size for such nutritional experiments, have an important agronomical interest and are close to human

physiology. We will describe these three methods, from the simplest to the most sophisticated ones. Here, we do not mention about studies which used parenteral nutrition to estimate nutritional requirements of the gut wall (Bertolo et al., 1998) as we believe they are outside the scope of this review.

2.1.1. Measurement of portal balance

One attempt to estimate the extraction of AA by the small intestine after absorption is to measure portal balance (PB). The principle of PB is to compare the difference in between AA concentration in the portal vein and the artery, relative to the digestible AA intake, over a given time period (Rérat et al., 1992). To this end, catheters must be placed in the vein and artery of the pig, to the measured AA concentration as well as blood flow (**Figure 8**). Most of the time, a continuous duodenal infusion of a very digestible protein sources (e.g., casein) was performed during the trial. Calculation was done in two steps, first the net portal balance (NPB):

$$(1)\ \textit{Net portal balance (NPB, µmol/kg/h)} = (Conc._{Port} - Conc._{Art}) \times PBF$$

Where $Conc._{Port}$ and $Conc._{Art}$ are the amino acid concentrations (µmol/L) in the portal vein and artery respectively and *PBF* is portal blood flow (L/kg/h). Fractional portal balance (FPB) can then be calculated by expressing NPB in percentage of AA intake:

$$(2)\ \textit{Fractional portal balance (FPB, \%)} = (\textit{Net portal balance/input}) \times 100$$

Where input correspond to the dietary AA intake (µmol/kg/h). The calculation of FPB provides an estimation of the percentage of dietary AA which can be found back in the portal vein after their absorption by intestinal cells. This method has the advantage to be relatively easy to but in practice; it requires two catheters to obtain blood samples and a flow probe to measure PBF. It is also not specific, meaning that it can be used for many AA simultaneously during an experiment. However, it is not an accurate measurement of first-pass metabolism. First, one of the catheters is located in the portal vein which drains blood from the SI has well as other splanchnic organs (**Figure 8**). Thus, it is an estimate of splanchnic first-pass metabolism, and not only intestinal first-pass metabolism. Second, this method does not allow to discriminate between AA from different origins, dietary or endogenous; thus, AA metabolised in first-pass cannot be distinguished from endogenous AA taken up from the blood. Finally, this method

considers splanchnic first-pass metabolism as a black-box and does not allow to go further in the metabolic use of AA by splanchnic tissue.

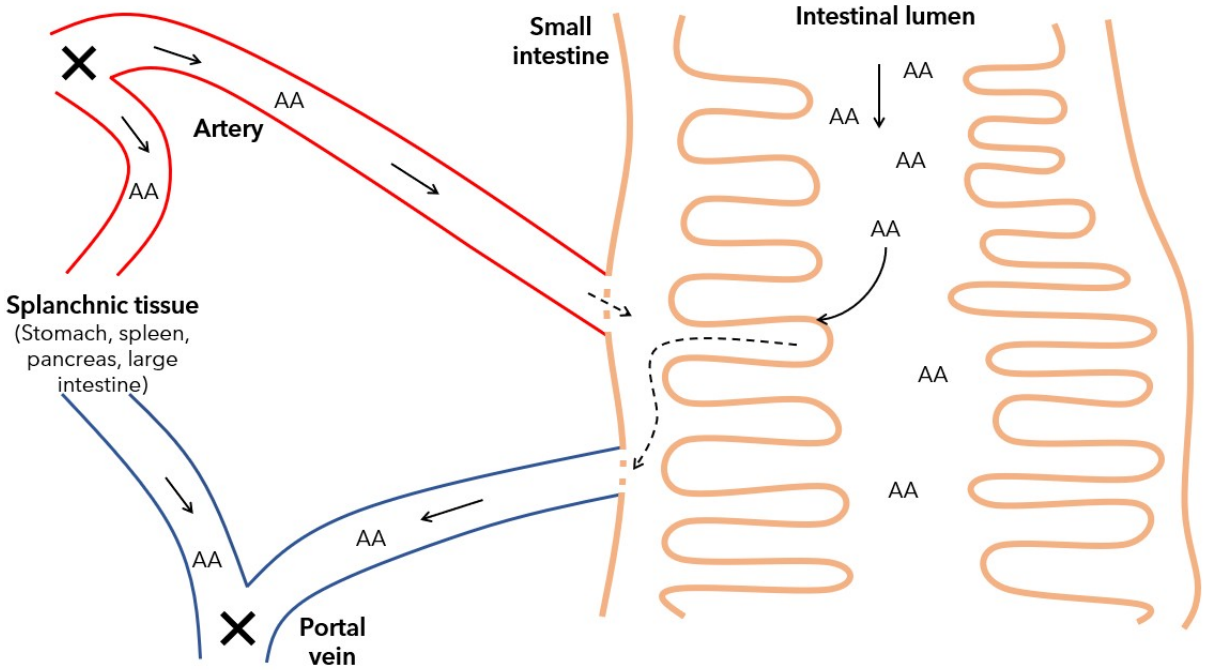


Figure 8. Scheme of the position of catheters used to estimate amino acid use by the small intestine of pig, *in vivo*.

2.1.2. Tracer portal balance

Based on similar principle as FPB, enterally administered tracers can be used to estimate first-pass extraction of AA (Reeds et al., 1996). Tracers are generally AA which are labelled with stable isotopes – often ^{13}C or ^{15}N. The principle as that a fraction of dietary AA is labelled (tracer) and has the same behaviour as the non labelled AA. With this method, calculation of tracer portal balance (TPB) lies on the same principle as NPB, but AA concentration in the vein and artery is multiplied to the tracer to tracee ratio:

$$(3)\ \textit{Tracer portal balance (µmol/kg/h)} = (Conc_{Por} \times t/T_{Por}) - (Conc_{Art} \times t/T_{Art}) \times PBF$$

Where t/T is the tracer-to-tracee ratio in the portal vein or artery. TPB can then be divided by the tracer infusion rate and multiplied by 100 to obtain fractional tracer portal balance:

$$(4)\ \textit{Fractional tracer portal balance (FTB, \%)} = (\textit{Tracer portal balance/Input}) \times 100$$

Where input corresponds to the infusion rate of the enterally administered tracer (µmol/kg/h). This calculation represents the percentage of the enterally administered tracer which is found back in the portal blood after intestinal absorption. The tracer portal balance method requires the same protocol as NPB, with the addition of the labelling of the AA of interest (often ^{13}C, ^{15}N), which is mixed with the rest of the infusion. This method allows to discriminate in between endogenous AA extracted from systemic blood and dietary AA, thanks to the tracer. One technical difficulty with this method is that it can be done for only one AA at the time, due to the stable isotope labelling. Finally, in this method the only input of tracer is considered to be the diet. However, in the SI, protein turnover is very fast, and AA can be metabolised by the SI before being released in the blood, rendering intestinal tissue a second source of tracer (Leterme et al., 1996).

2.1.3. Dual tracer balance

This method combines the infusion of traced AA in the digestive tract, flowed by the infusion of a traced AA in the blood, or the opposite (van Goudoever et al., 2000). For both infusion, stable isotopes, often ^{13}C labelled AA are used. Both infusions are performed over a certain time period on the same animal, with measurements in the artery and vein, as well as portal blood flow. The use of the same label for each infusion leads to necessity of separating each period of infusion by one or two days, to avoid confusion in the origin when looking a tracer recovery. The first step in the series of calculations consist in calculating the fraction of parenterally administered tracer used by the intestine during the infusion:

$$(5)\ Arterial\ input = ConcPar_{Art} \times PBF$$

$$(6)\ Portal\ output = ConcPar_{Por} \times PBF$$

$$(7)\ Fractional\ arterial\ use\ (\mu mol/kg/h) = (Arterial\ input - Portal\ output)/Arterial\ input$$

Where *ConcPar* is the concentration of the parenterally administered tracer in the vein or artery. Equation n°7 allows to estimate fraction of arterial AA that is used by the intestine. Then, fractional enteral AA use by the SI can be calculated as:

$$(8)\ Arterial\ input = ConcEnt_{Art} \times PBF$$

$$(9)\ Portal\ output = ConcEnt_{Por} \times PBF$$

(10) Fractional enteral use (µmol/kg/h) = Dietary tracer intake – Portal output – [(1-Fractional arterial use) × Arterial input]/Dietary tracer intake

Where *ConcEnt* is the concentration (µmol/kg/h) of enterally administered tracer in the vein or artery and dietary tracer intake is expressed in µmol/kg/h. This can later be converted to dual tracer balance (DTB):

(11) DTB (%) = 100 – (fractional enteral use × dietary intake)/dietary intake

This method of calculated has the advantage of differentiating explicitly AA use from systemic or dietary origin by the SI. To the author's knowledge, is the most accurate measurement of AA first-pass metabolism by the SI which can be obtained *in vivo*. However, due to the position of catheters in the blood, it is rather a measurement of AA at the level of the PDV than only the SI. This method is obviously the most difficult to put in practice, has experiments have to be performed with labelled AA over two time periods. Contrarily to other black-boxes approaches present before, this method allows the calculation of all the fluxes identified by a letter in **Figure 9.** Hence, it allows a more precise estimation of the metabolic fate of AA in the SI. It is to note that to determine AA oxidation, $^{13}CO_2$ concentration in the blood must be measured. Rapidity at which metabolic process occur in the SI does not allow with this method, to determine if a dietary AA appearing in the blood has been previously metabolised by the SI or not (Leterme et al., 1996).

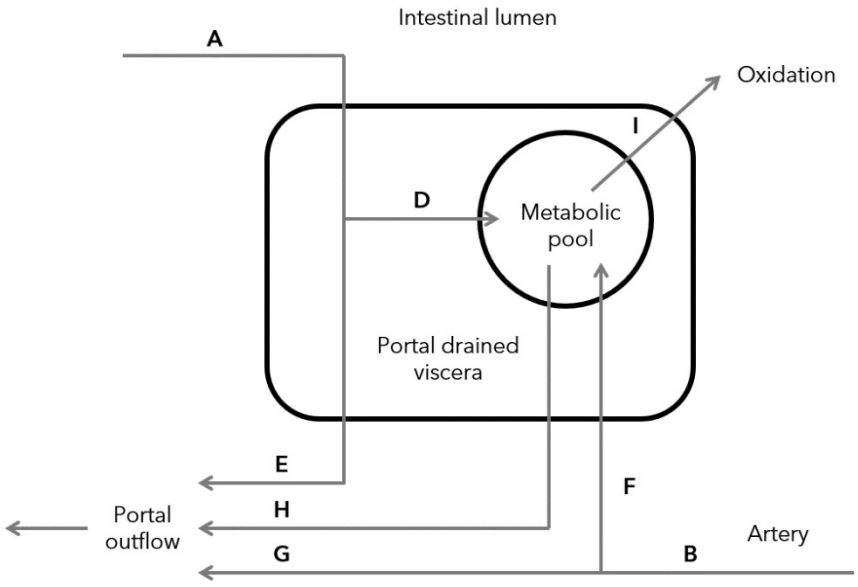


Figure 9. Amino acid fluxes at the level of the small intestine which can be estimated using the dual tracer balance method. Each letter designates a flux which can be quantified with this method. Adapted from van Goudoever et al., (2000).

4.3. A quantitative analysis of results

In a previous study conducted during an MSc thesis, results published in literature using the three above mentioned methods to quantify AA intestinal metabolism were compiled and analysed (Garçon et al., 2021). From this it was concluded that intestinal use of AA by intestinal cells is highly variable and depends on a wide range of parameters that could not be identified due to the small number of studies which have been performed (19). Results focusing on NPB balance for EAA, from 9 studies, showed that on average ≈ 60% of digestible dietary EAA were found in the blood during digestion (**Figure 10**). For NEAA, there was much more variation among AA, with NPB ranging from 0% or negative for glutamate and glutamine to more than 150% for alanine. Even by taking animals in similar physiological range, with the use of DTB method, reported values for the use of intestinal AA were highly variable between studies. As an illustration, for lysine first-pass as estimated by DTB method in three studies ranged from 0 to 55%. It seems that many factors influence first-pass use of AA, and that these *in vivo* studies did not allow to obtain a precise quantification.

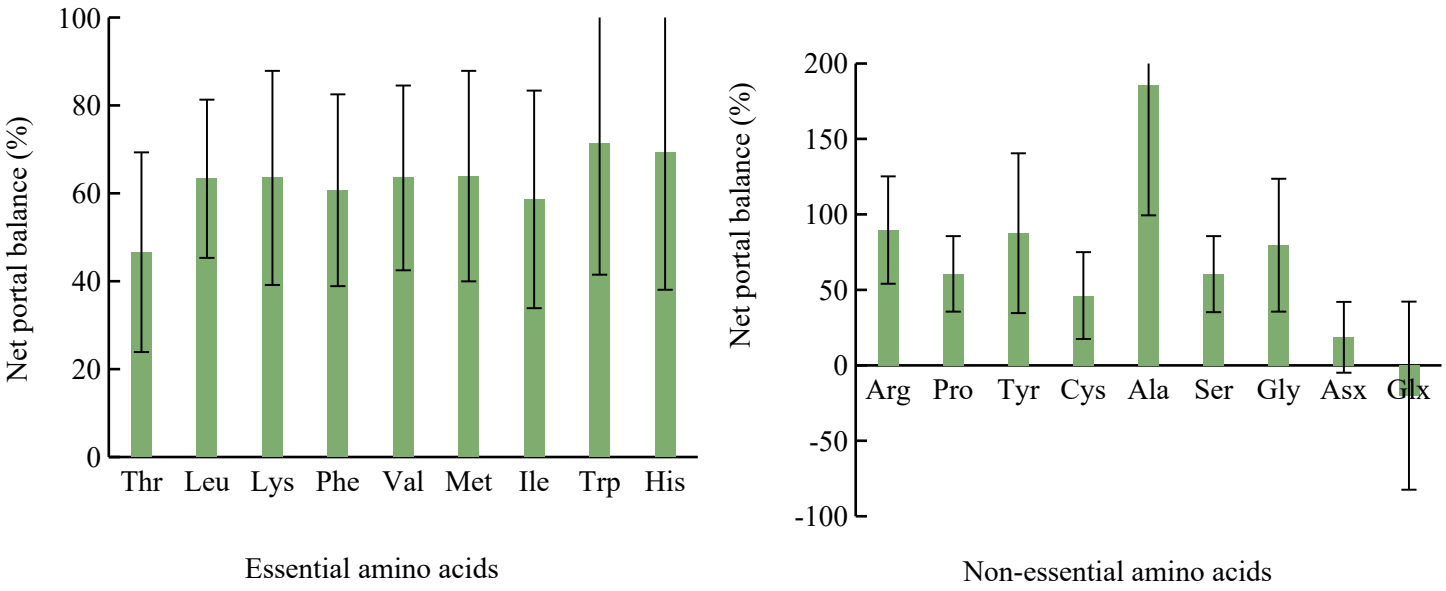


Figure 10. Net portal balance as expressed in percentage of intakes of digestible, for essential and non-essential amino acids (n=9). Results originate from Garçon et al., 2021.

4.4. Conclusion on estimations of amino acids fluxes in the small intestine *in vivo*

The concept of first-pass metabolism originates from pharmaceutical studies and has later been in nutritional studies. Studies attempting to quantify intestinal use of AA using catheterised pigs have been performed using three different methods, offering more or less accuracy. Quantitative analysis of results published in literature showed a high variation in AA use by the SI, both in between AA and studies – regardless of the method used. Therefore, it seems that *in vivo* methods do not allow to obtain a quantitative estimate of first-pass metabolism. They also do not provide insight on the metabolic fate of AA inside intestinal cells. Finally, *in vivo* experiments to quantify intestinal metabolism rise ethical concerns; thereby new methods of investigation should be evaluated.

5. The use of mathematical models in animal science and nutrition

5.1. Definition of mathematical models in biology

The term modelling encompasses a large variety of methods, from the use of a statistical linear regression model to estimate the effect of a treatment, to mechanistic models or neural network models. All these tools use different mathematical and computational methods, with different outcomes and aims. They have in common of being a simplified representation of a reality, based on mathematical equation. Classification of models in biology can be done with regards to different aspects, namely optimisation method, application, time, behaviour and natural context (Tedeschi and Menendez, 2020). Here, I will mainly focus on dynamic, deterministic, mechanistic models. These models are made of a set of differential equations, representing the behaviour of a biological system. They require a conceptualisation of how the system works and how elements are connected to each other (France, 1988). The term mechanistic indicate that these models rather focus on the relationship between elements and the way they interact, than numerical predictions.

5.2. A brief history of the use of mathematical models in animal science and nutrition

It seems that the development of mathematical models in nutrition came from the area of animal science, with the need of determining the requirement of animals and the fate of dietary nutrients (Dumas et al., 2008). First aspects modelled in animal science were related to animal growth, energy requirements in the early 19th century (Armsby, 1903; Brody and Ragsdale, 1921). These first models were developed toward the idea of prediction. One of the first study using a modelling approach to investigate a nutritional question was from Erwing and Smith (1917), who estimated digesta flow in steer to predict digestion coefficients. Later, the model of Blaxter et al. (1956) was one of the first to use differential equations to model digesta flow, in sheep. Blaxter et al. (1956) were among the first to come up with the idea of including different compartments (rumen, abomasum, duodenum, faeces) in their mathematical representation of the gastrointestinal tract.

The onset of the use of mechanistic models in animal science really started from the 1970s, probably due to the increasing availability of computers to perform calculations. This period coincides with the increase in use of compartmental model in animal science, made of series of

differential equations. Scientists developing these models were not focused on prediction *per se*, but rather to integrate current knowledge and gain understanding on underlying processes when studying a biological phenomenon. This was the case for the model of Baldwin and Smith (1971), which represented fat metabolism in ruminant or Neal and Thornley (1983) who described milk production by the mammary gland.

One of the first model integrating processes at the scale of the whole animal was from Black (1984), simulating energy and AA use by the pig. It was later followed by other models such as the one developed by NRC (2012) or by INRAE (van Milgen et al., 2008). Modelling of digestive processes also gain increasing importance, especially the rumen received much attention and is still an area of important research nowadays (France et al., 1982; Dijkstra et al., 1992; Lingen et al., 2016). One of the first mechanistic model to integrate all aspects of digestion was developed in pig by Bastianelli et al. (1996). This model was rather an attempt to integrate current knowledge and identify areas of further research in nutrition than prediction *per se.*

In the coming years, at the scale of the animal or the herd a lot of synergies between mechanistic models and data driven models are anticipated thanks to the use of sensors (Ellis et al., 2020; Gaillard et al., 2020). For studies about metabolism or digestive physiology, it is likely that amount of data available will often be limiting data driven approaches; thus, other way forwards must be found. Schop et al. (2023) recently proposed a model of digestion in pig, integrating physico-chemical properties of digesta, obtained with *in vitro* experiments. This combination of *in vitro* and *in silico* approaches could be a way forward for mechanistic models in the area of nutrition. It could allow to obtain new parameters to integrate in models which are difficult to estimate using *in vivo* studies (e.g., rate of transport).

While modelling approaches are widely used in nutritional science for animals or pharmaceutics, less research has been performed in relation to food digestion or post postprandial metabolism in human (Poupin et al., 2014; Rivera del Rio et al., 2022). It might be because digestive processes and nutrition in human is a more recent area of investigation and many of its progress have been possible through the use of animal models, such as pig or murine model. Research on human nutrition continue its development, and reductions of animal experiments are likely to push scientist toward more *in silico* approaches, which could also

complement the widely developed *in vitro* methods (Brodkorb et al., 2019; Colombo et al., 2021).

5.3. Models of digestion

3.1.1. Holistic models of digestion

Several models have focused on describing digestive processes occurring in the lumen of gastrointestinal tract, using a holistic approach. The term holistic signifies that these models intend to include all (or almost) processes of digestion happening along the gastrointestinal tract. In particular, two similar models representing nutrients digestion and absorption in the gastrointestinal tract of pigs have been developed (Bastianelli et al., 1996; Strathe et al., 2008). More recently, a model was developed, based on human physiology, describing protein digestion and investigating the influence of physical state of the meal (Rivera del Rio et al., 2022). Data from several studies were used to parametrise the latter model and outputs were compared to the results from one selected study. All these models have in common to integrate current knowledge on digestive processes and were used to unravel mechanisms which are difficult to study *in vivo.* Another attempt to integrate all processes of food digestion in the digestive tract was performed by van Aken, (2024) in human. This model was constructed with the aim of predicting postprandial physiological markers based on digestive physiology, as an alternative to *in vivo* experiments. The main disadvantage of holistic models such as the latter, is that numerous processes are integrated which cannot be all described in one article. Thus, potential users of these models cannot understand the underlying principles and assumptions on which they are based; which hampers the ability to understand models' outputs. The attempt to represent explicitly many processes also generates the risk of overcomplexity of the model, with too many parameters and assumptions which cannot be verified. One attempt to overcome this issue and obtain required parameters was performed by Schop et al., (2023) who used *in vitro* experiments to estimate physio-chemical properties of digesta and implemented them in a model of digestion in pig. Overall, these holistic models can be used to better understand how all digestive processes interact together, investigate specific mechanisms or understand experimental results. Their use for prediction and as alternative to animal experiments in nutrition is not yet feasible.

3.1.2. Representation of the small intestine in models

One of the main difficultly with the SI is that it's a long tube, along which digesta transit, is mixed, progressively digested and nutrients are absorbed (Lentle and Janssen, 2008). Besides, there are some structural and functional differences along the SI (Raul et al., 1988; Boudry et al., 2004; Wang et al., 2009). This is different from the stomach, the rumen or the large intestine which are often represent as one homogenous batch in which digesta is mixed and degraded. Some models have in common to split the SI in two segments, while other anatomical compartments (stomach and large intestine) are composed of just one (Bastianelli et al., 1996; Rivest et al., 2000). This representation allows to differentiate parameters from one segment to another, thus explicitly representing regional differences in the SI. However, these compartments behave like two batches connected to each other, which is not the behaviour expected from the tubular structure of the SI. In a study performed by Strathe et al. (2008), the SI was divided in 50 segments, varying in length. At regular time intervals, a segment empties in the following one, mimicking the continuous flow of digesta through the SI. This representation allowed to capture the tubular structure of the SI. In this model, size of the segments decreased progressively toward the distal part of the SI. This allowed, to have a retention progressively reduced along the length of the SI. An intermediate approach was proposed by Rivera del Rio et al. (2022), who represented the SI with seven compartments, inspired by the work of Yu et al. (1996). This was based on morphological considerations, the first compartment being duodenum, second and third, jejunum and fourth to seven, ileum. A singular approach was developed by Taghipoor et al. (2012) to simulate transit and degradation of the digesta along the SI. This model is a more accurate representation of movements of the digesta due to peristaltic movements of the SI. Interestingly, it is not the SI that is explicitly represented but rather the bolus moving along an axis. The downside of such model is that its mathematical formalism is complex – rendering it difficult to use by biologists.

The area of pharmaceutical research also produced several models, representing drug absorption in the SI (Sjögren et al., 2016). Interestingly, these models in addition to representing processes occurring in the intestinal lumen, also represent explicitly absorption at the level of the mucosa and first-pass metabolism (Sjögren et al., 2013). The idea behind these models is to be able to use them for drug development; thus, being able to predict the availability of an orally administered drug.

5.4. Models of post-absorptive metabolism

Models of post-absorptive metabolism in animal science focus on processes such as animal growth (van Milgen et al., 2008) or nutrients use by the mammary gland (Hanigan et al., 2001; Krogh et al., 2021). Models of animal growth consider nutrients available for the organism after digestive processes and use standardised ileal digestibility (SID) of nutrients as input (NRC, 2012; van Milgen et al., 2008). Standardised ileal digestibility is an estimation of the amount of nutrients absorbed at the end of the ileum, corrected for basal endogenous losses (Stein et al., 2007). Basal endogenous losses being the sum of endogenous protein and AA found at the end of ileum during digestion, not influenced by feed characteristics (presence of anti-nutritional factors, fibre…etc.). Which means that when using SID, the interplay between the digestive tract and the feed is partly accounted for. However, it also means that endogenous losses due to feed characteristics (specific ileal losses), which might represent a metabolic cost for the organism, are not considered in most of models of post-absorptive metabolism. Besides, by using SID as an input, these models consider that material which disappears from the intestinal lumen appears in the blood. Yet, intestinal metabolic activity, which is high, as mentioned above, can modify the profile of AA appearing in the blood, which will ultimately be available for growth. These models consider a maximum efficiency of AA for protein deposition, in the range of 0.75 (NRC, 2012; van Milgen et al., 2008). This means that at least 25 % of SID AA are inevitably catabolised by the organism and not available for weight gain. These inevitable losses could be partly due to metabolic activity of the SI.

5.5. A bridge between digestion and post-absorptive metabolism

While being the interface between digestion and post-absorptive metabolism, intestinal metabolism has received few attentions by scientists attempting to model digestive and post-absorptive processes in nutritional science. The idea of integrating aspects of GIT wall in models of digestion was already introduced by Bannink et al. (2006) for farm animals and more recently in human Le Feunteun et al. (2021). A model specifically representing processes at the level of intestinal mucosa was developed by Mills et al. (2017) for starch digestion and subsequent glucose absorption by the SI in dairy cow. This model represented processes ongoing at the level of the unstirred water layer, as well as inside intestinal mucosal cells. This is to our knowledge, the only model in nutritional science giving a specific emphasis to these

aspects. The downside of such approach is that for processes ongoing at the level of the unstirred water layer or inside mucosal cells, data to quantify phenomenon and parametrise equations are scarce. Biologists often see the interest of modelling in a predictive manner, thus considering that the presence of data is a cornerstone to develop a model. Yet, in system science, data have been considered the least important thing when developing a model (Forrester, 1980). Stressing the fact that to describe the relationship between elements or the functioning of a process, it is rather the elements to consider and the construction of an equation that matters than exact parametrisation. Obtaining accurate data allows to obtain more accurate outputs, but it does not change the direction of cause to effect relationship between variables.

5.6. Conclusion on mathematical models

Mathematical modelling encompasses a large variety of methods which every biologist uses nowadays, from a simple linear regression to artificial intelligence-based models. Here, we mainly focus on models which are dynamic (over time), deterministic (no random variation), and mechanistic (cause to effect). These models rather focus on the relationship between elements from which they are composed and the way they interact, rather than numerical predictions. In models of digestion, representing the SI has often been a challenged, due to its tubular structure. Most of the time it is divided in segments, ranging from 2 to 50, sometimes based on morphological differences. The SI, which is at the interface between digestive processes and post-absorptive metabolism as received few attentions in the modelling community. From our knowledge, one model from Mills et al. (2017) has attempted to explicitly focus on processes occurring in the intestinal mucosa, for glucose absorption in dairy cow. Most of biological processes ongoing in the SI are known, although some data might be lacking for model parametrisation. However, to construct a model, this should not be limiting if we consider that the model is rather focused on describing relationship between elements *via* the selection of appropriate elements and equations.

6. *In vitro* methods to study intestinal amino acid metabolism

6.1. *In vitro* models of intestinal cells

The first method to cultivate intestinal cells *in vitro* was the use of isolated intestinal cells initiated by Watford et al. (1979) with cells from rats and chicken. It was later widely used by the group of Wu et al. (1994) in the 1990s, which performed numerous studies using piglets isolated intestinal cells. The principle of this method is to harvest mucosal cells from the intestine of an animal which was slaughtered, and then cultivate them in a medium. The particularity of intestinal mucosal cells is that they are adhesive cells which tend to attach. Often, for trials these cells were cultivated for relatively short periods around 30 minutes, in flasks or tubes, sometimes placed within a shaking water bath (Wu et al., 1995; He and Wu, 2022). Often, isolated cells are termed enterocytes in these studies (Wu et al., 1994), yet the process of harvest does not allow to discriminate on between the different cell types of the mucosa. The downside of such method is that harvested cells must be used very quickly and cannot be kept more than 24-48 hours as reviewed by Randall et al. (2011). However, this allows to have a sample of mucosal cells which has a cell population coherent in comparison with *in vivo* intestinal cells. In addition, cells from different morphological segments or physiological status can be used and compared. However, sampling procedure on different animals can be a source of variation, and impair the reproducibility of the results. **Figure 11** sums up main properties of several intestinal cell models, including isolated intestinal cells.

Commercial cell lines have been also widely used to study the SI, since the 1980s, with the most commonly found being Caco-2 (Pinto et al., 1983). The idea of the use of Caco-2 cells on transwells to mimic polarisation of the SI dates from nearly forty years ago (Hidalgo et al., 1989). These cells have the advantage to be relatively easy to keep in a laboratory set-up and to multiply. Caco-2 cells can be incubated over very long periods of time, up to a month. They also offer a good reproducibility, due to their identical origin. However, their relevance to mimic the behaviour of epithelial cells from the SI can be questioned. In fact, Caco-2 cells are carcinoma cells originating from human colon; thus, they are probably different from small intestinal mucosal cells from a healthy individual. For example, it is considered that colonocytes do not exhibit the ability to transport free AA from the lumen, contrarily to enterocytes (van der Wielen et al., 2017). In addition, Caco-2 cells are composed of only one cell type

(colonocytes), while several are present in the SI mucosa. To overcome this issue, these cells can be co-cultured, for example with mucus (Hilgendorf et al., 2000) or immune cells (Ding et al., 2021). Due to their well-known culture conditions, these cells are relevant models for screening or testing the effect of a molecule on the intestinal barrier, and obtain comparable results in between laboratories. This usage have been common in the past decades, especially in pharmaceutical research (Meunier et al., 1995). Culture conditions of Caco-2 cells can be modified, for example to mimic inflammation of the intestinal mucosa (Wu et al., 2019); stressing the versatile potential of these models. Recent efforts in the use of intestinal cell lines and especially Caco-2 cells are in the direction of harmonisation of culture conditions (Faria et al., 2024).

Organoids are the most recently developed intestinal cell models, and their use for farm animal has just started as reviewed by Beaumont et al. (2021). Organoids are self-organised three dimensional spheric microstructures formed of a monolayer of polarised intestinal epithelial cells, surrounding a hollow lumen. They originate from intestinal stem cells harvested from the crypts of small intestinal mucosa on animals. These stem cells being sampled and cultivated in culture medium with diverse developmental factor to grow and differentiate in organoids over several days. The main advantage of organoids is that they offer *in vitro* models comprising most of cell types which can be found in the intestinal mucosa. However, one difficulty for their use as absorptive models is to obtain a two dimensional cell monolayer from their initial three dimensional structure, but protocols are being developed (Mussard et al., 2023). Due to the fact that organoids originate from a donor animal, some properties of the cells can be retained such as the phenotype associated to the gut region (Mussard et al., 2022). However, this could also be a drawback because different origin of organoids might create more variability and less reproducibility in results obtained. While being a very promising model, intestinal organoids is still a method under development, and progress in their culture technique is necessary to facilitate the spreading of their usage among laboratories.

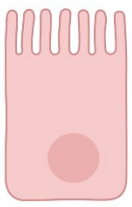
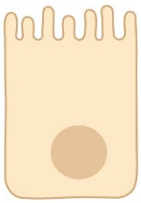
	Isolated intestinal cells	Commercial cell lines	Intestinal organoids
			
Origin	Harvested from any animal (epithelial cells)	Biobanks Human carcinoma from colon (Caco-2) Unsuckled enterocytes from piglets (IPEC-J2)	Harvested from any animal (stem cells from crypts)
Culture duration	Short duration (24-48h) Already differenciated	Generally for 21 days Differenciation needed	0 to 20 days Differenciation needed
Support	Wells (transwells)	Wells, transwells or chip	Wells, transwells or chip
Cell types	Mucosal cells	Enterocytes (Caco-2, IPEC-J2) Goblet cells (HT29-MTX) Possible co-culture	Any epithelial cells Possible co-culture
Structure	Adherent cells	2D cell monolayer	3D spheric with a lumen (can be converted in 2D monolayer)

Figure 11. Comparison of the main characteristics of three *in vitro* models of intestinal cells.

6.2. Focus on the IPEC-J2 cell line

Research on the database Web of Science using IPEC-J2 as a key word in the field "topic" leads to 1,100 results while it is 27,400 for Caco-2. Most of the results for IPEC-J2 are from the last ten years, showing a growing interest for this intestinal cell model. This cell line has been isolated by Helen Berschneider at the university of North Carolina (USA) and first used to investigate ion transport and enterocytes differentiation (Berschneider, 1989). First publications using IPEC-J2 cell line already dates from the 1990s (Kandil et al., 1995), but there usage in research has remained much more confidential than Caco-2 cells. It is a non-transformed, permanent cell line isolated the jejunum of neonatal unsuckled piglets (Vergauwen, 2015). Contrarily to the Caco-2 cells these cells are thus non-cancer and originate from the small intestine; rendering them a promising model for the study of SI. To my knowledge, only a few studies have been published on the characterisation of the IPEC-J2 cell model. Two studies on the characterisation of this cell line found that IPEC-J2 cells possess microvilli on their apical surface, are attached together with tight junctions proteins (claudins and occludins) and express

glycocalyx-bound mucin (Schierack et al., 2006; Zakrzewski et al., 2013). These elements suggest a polarised cell monolayer retaining the main features of the intestinal epithelium. Another study from Pi et al. (2022) studied gene expression and enzymes activity in cultivated IPEC-J2 cells. This study showed an increase in the alkaline phosphatase activity – a marker of enterocytes differentiation – after 14 days, suggesting that these cells have the ability to differentiate in laboratory culture conditions. Such results would need to be confronted to *ex vivo* sampled intestinal cells to assess the similarities in between IPEC-J2 cells and intestinal mucosal cells. This cell model have been mainly used to investigate the barrier function of intestinal epithelium in different conditions (Yan and Ajuwon, 2017; Tretola et al., 2021; Perruchot et al., 2025). They are potentially an interesting tool to study small intestinal epithelial cells in laboratory conditions.

More work on the characterisation of this cell model is required, on aspects such as cell types present, the presence or absence of mucus layer, expression of transporters, similarity with the phenotype of pig's enterocytes… These elements are important for a better interpretation of results obtained later when using IPEC-J2 as an absorptive model. Efforts to homogenise culture conditions are probably a second step required to develop the use of this cell line and allow comparison of results obtained in between laboratories. To my knowledge, three studies have been performed in order to optimise culture conditions of IPEC-J2 cells. One focused on parameters such as TEER, histological observations and electron microscopy at different time points and with varying seeding densities (Bernardini et al., 2021). Another assessed the effect of different concentration of foetal bovine serum (FBS) on mitochondrial activity of the cells (Geens and Niewold, 2011). Another investigated the culture of IPEC-J2 cells with different culture mediums and concluded that the use of porcine serum (PS) allowed to obtain properties closest to pig's epithelial intestinal cells (Zakrzewski et al., 2013). It can be expected that in the future, efforts to homogenise culture conditions of IPEC-J2 cells and a better characterisation will occur, as for Caco-2 cells (Faria et al., 2024).

6.3. Cell culture methods

Several culture methods are available for intestinal cell and can be divided in three main types which are the use of wells (or tubes), transwells and on chips (**Figure 12**). The most simplistic method is the use of well, which are often in plates containing usually from 6 to 96 wells; but

the principle is the same with the use of tubes or flasks. This was the first method used for culturing isolated intestinal cells (Watford et al., 1979). In these conditions, intestinal cells which are seeded attach to the bottom and are immersed in a culture medium. In some situations, the culture is placed in a shaking water bath to maintain temperature and allow homogenous mixing of the medium (Wu et al., 1995). One single cell type can be cultured or several, without spatial separation in between them. Transwells have then been developed, allowing to obtain a polarised layer, with an apical and basolateral side. Cells are seeded and attach to a porous membrane which separates an apical and a basolateral chamber. Using this set-up, co-culture can also be performed and a spatial separation can be performed if one cell type is seeded in the basolateral chamber and another in the apical one (e.g., absorptive and immune cells). Usually, transwells can be directly added to 6 or 12 wells plates. The last method of cell culture which can be used for intestinal absorptive cells is the organ on chip (or chips), which is at an early stages of development (Zhang et al., 2018). Many potentialities can be seen with such set-up, with the presence of a fluidic flow to mimic digesta or blood flow, the combination of cell types over a defined organisation or mechanical stimulation. Yet, only some pioneering studies have been performed with these devices and several years are probably required for the spread of their use in nutritional studies.

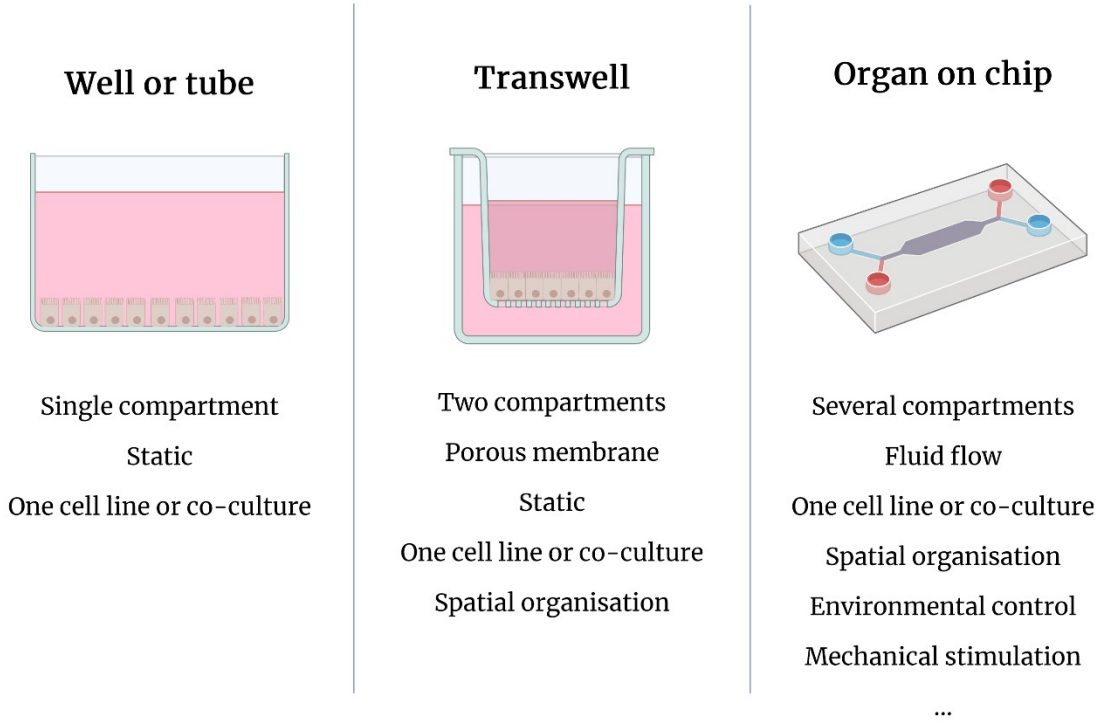


Figure 12. Three possible *in vitro* culture techniques for intestinal cells.

6.4. Use of cell models to investigate amino acids metabolism

The first use of isolated intestinal cells was done in order to investigate their metabolic use of several substrates, including AA, as well as release of nitrogenous products (Watford et al., 1979). Several studies focusing on AA use by intestinal cells, presence of metabolic pathways and measurement of the amount of product released were performed by the group of Wu et al. (1995) in the 1990s using piglet's intestinal cells. These studies were performed over a relatively short period of time (30 min), with isolated intestinal cells immersed in a buffer media, with the addition of a metabolic substrate (e.g., glutamine). Production of metabolic products or O_2 consumption could then be measured in controlled conditions. These studies allowed to assess the preference of intestinal cells over different energy substrates as well as the presence or absence of metabolic pathways at different developmental stages. Labelling with ^{14}C isotope have been sometimes use to determine the fate of carbon chain from labelled AA in other metabolites or CO_2 (Wu, 1997). Measurement of enzymes activity was also performed to assess the importance of metabolic pathways (Wu et al., 1994). Although

providing insight on AA metabolism by intestinal cells, these studies did not replicate the organisation of the intestinal epithelium – as a polarised monolayer of cells.

The use of commercial cell lines, such as Caco-2, has often been restricted to transport studies (Hidalgo and Borchardt, 1990), epithelial barrier function (Wu et al., 2019; Arranz et al., 2023) or interactions with microorganisms (Finlay and Falkow, 1990). One study used IPEC-J2 cells with or without arginine to investigate the effect of arginine on intestinal epithelial cells and its mode of action (Bauchart-Thevret et al., 2010).

One interesting study from Sakai et al. (2020) investigated the metabolic use of several AA by a polarised layer of intestinal cells. They used Caco-2 cells seeded on transwells and incubated for 24 hours, with an AA rich medium containing glutamate labelled with stable isotopes (^{13}C,^{15}N) in the apical side. Then, samples of apical and basolateral medium were taken and analysed for the isotopic enrichment. This method allowed to estimate passage of apical AA through the epithelial barrier as well as measurement of the fate of ^{13}C and ^{15}N from glutamate, over time. Yet, measurements were performed over a long period of time (24h), which is not representative of processes happening during digestion in the SI, where transport happens in a range of a few minutes, when digesta in in contact with the epithelium during its transit. In addition, a large shared of ^{13}C (87 %) and ^{15}N (40 %) was not found back at the end of the experiment, suggesting either volatilisation in the form of CO_2 or NH_3, or incorporation into intestinal cells. In one study with bovine mammary epithelial cells, Yoder et al. (2020) performed a similar approach, with labelled AA and cells incubated in wells. They used two kinds of marker, one after the other to assess bidirectional fluxes, from the medium to the cells and the cells to the medium.

6.5. Conclusion on *in vitro* culture of intestinal cells

Different types of *in vitro* intestinal cell models can potentially be used, with different culture set-up. They vary in term of complexity and the extent to which their culture conditions in a laboratory are mastered. It seems that the use of commercial cell lines offers the advantage of simpler cell models to cultivate with well controlled culture conditions. Among cell lines, the IPEC-J2 cell line is a promising model, which fits with the aim of studying SI cells from pigs. In fact, they are non-transformed jejunal cells from unsuckled piglets. However, they have been mainly used to study the barrier function of the SI until now. The use of IPEC-J2 cells cultivated

on transwells to study metabolism of AA could be a way forward. However, studies which focused on AA metabolism in intestinal cells *in vitro* in a dynamic manner are scarce and such protocol would need to be developed.

7. Knowledge gap

The present thesis was initiated by the intuition that small intestinal metabolism of AA has strong quantitative influence of AA appearance in the blood during digestion. As reviewed, *in vivo* studies have tried to investigate influence of intestinal metabolism of AA on portal appearance of AA, with the underlying concept of first-pass metabolism. First-pass metabolism (or extraction) derives from pharmacologic studies and represent the extraction of a compound before reaching its target organ. In the case of intestinal first-pass metabolism of AA, it represents fraction of dietary AA metabolised by the intestinal tissue after absorption, thus not appearing in the blood. Several research groups used catheterised pigs to investigate at the fraction of digestible AA found back in portal blood after digestion, sometimes using labelled AA. Only a small amount these studies (< 20) have been performed and their results – although highlighting the importance of AA intestinal metabolism – do not allow to quantify first-pass metabolism. One of the drawbacks of these studies is that the placement of catheters does not allow to discriminate AA taken by the SI from those taken by the PDV, in the blood. In addition, rapidity at which metabolic processes occur and AA are recycled complicates the estimation of intestinal use. Other aspects, such as length of the SI or the presence of numerous cell types performing different functions further complicates the quantification of fluxes of AA *in vivo*. The aim of these studies was to quantify AA use by the SI, but they did not investigate metabolic processes occurring inside small intestinal cells – which would explain the measured fluxes. Intestinal metabolism of AA goes beyond first-pass metabolism, as an illustration, endogenous protein secretions also interact with digestive processes and measured digestibility of AA. In short, metabolic processes in the SI are not a simple disappearance of AA and suggests complex metabolic activity interacting with digestive processes – which require further investigation.

New methodologies should be used to explore and gain insight on intestinal metabolism of AA. The review above mentioned the importance that had mathematical models in animal science and nutrition. Yet, models are often split in two categories, models of post-absorptive metabolism and models of digestion. The SI which is at the interface of both as received few

attentions for now. With regard to cell models, numerous methods and cell types can now be cultivated to study intestinal metabolism. Yet, for now they have not been used to study AA metabolism in a dynamic manner, over a polarised layer of intestinal cells. Such approach could be relevant to complement a mathematical modelling approach.

8. Research approach

The literature review highlighted the limits of *in vivo* approaches to quantify metabolic use of AA by the SI. One additional point with regard to such experiments using catheterised pigs is that they are now limited due to ethical concerns. Thereby, we sought to develop research methodologies which would provide insight on quantitative AA metabolism in the SI, without the use of animal experiments. The overall aim of this thesis was to better understand AA metabolism in the SI and its interactions with digestive processes. To this end, a combination of two complementary approaches was chosen, which where the construction of a mathematical mechanistic model (*in silico*) and cell culture (*in vitro*). Another aim of this thesis was to assess whether a combination of approaches which are alternative to animal experiments are relevant to study digestive physiology and metabolism. Motivations behind the choice to use both these *in silico* and *in vitro* approaches to quantify AA metabolism of AA are presented below.

In the modelling approach, the aim was to construct a mathematical model representing metabolic fluxes of AA in the SI. The construction of a mathematical model of intestinal metabolism of AA requires to integrate current knowledge available, which have been partly summed up above. This allows to aggregate and put in perspective different fraction of knowledge which have been studied separately and assess how they interact with each other. As an illustration knowledge acquired from previously *in vitro* and in *vivo* studies – at different scales – could be combined together. Model construction is also a way to explore the functioning of a biological system, which is the SI in my case. In *in vivo* studies with pigs, the SI was often taken as a black box, looking at digestible AA available in the lumen and AA appearing in the portal blood. A model would allow, based on current knowledge, to reconstruct the steps which happen in between to better understand and explain results obtained *in vivo* and their driving forces. Finally, model's constructions allow to test hypothesis about the functioning of processes within the biological system. As an illustration, we emphasised above that although there is no direct *in vivo* measurement, it is estimated that most of AA are

transported via saturable transporters. The effect of such hypothesis on functioning of intestinal metabolism could be investigated in a model.

In attempting to model AA in the SI during digestion, choices must be made about which elements to integrates or not. Numerous elements have been highlighted in the literature, and relevant ones in view of quantifying AA metabolism in the SI need to be identified. Another important step is to represent the relationship between elements of the model, by the appropriate mathematical equations. This consists in identifying the driving forces for each process. Finally, parametrisation of the model is the last step of its construction. Some parameters can be found from data in the literature, but it is likely that many of them will not be available. We believe that this should not be seen as a limit for the effort of modelling SI metabolism, as the first two steps, choice of appropriate elements and identification of the driving forces are the most important steps in the process.

It is in this context of a lack of data for model parametrisation that came the idea of an *in vitro* approach, to complement the scientific approach of this thesis. In fact, cell models could allow to obtain data lacking for model parametrisation and which cannot be obtained *in vivo*. Cell culture could also be a way forward to assess the cause to effect relationship in between elements of the model, in view of choosing the appropriate equations. *In vitro* cultured cell models allow to study the metabolic functioning of a subset of intestinal cells, in fixed and known conditions. As for the mechanistic model, it is a simplification of a real biological system, which is the SI. It is a more specific approach, in the sense that it allows to study the behaviour of a subset of epithelial cells, while the computer model is at the scale of the SI. Cells models are now widely developed and used since a long time to study the use of AA by intestinal cells. The potential use of *in vitro* cultured intestinal cells as a polarised cell layer with a dynamic approach over time has, to our knowledge, not been explored for now. While these are two important features of intestinal metabolism which we aimed to integrate in the cell culture set-up. The IPEC-J2 cell line, which originates from piglet's jejunum seems an ideal compromise in between proximity with the object of study (the SI) and the ease to cultivate in a laboratory set-up. IPEC-J2 cells have the advantage, as a cell line, to be relatively easy to cultivate and provide a good replicability. These cells grow and differentiate over several days, which allows to grow them on transwells, forming a polarised layer, with an apical and basolateral side. Two AA which are differentially metabolised by the SI, namely glutamine and

lysine were chosen for these experiments. It is expected that differences in the metabolic use of glutamine and lysine should be observed in cell culture conditions, if they maintain features of small intestinal cells. To follow their kinetic of use, they were uniformly labelled using stable isotopes (^{13}C, ^{15}N).

The experimental approach of this thesis can be divided in two main parts, according to the methodology used:

- The *in silico* part of this thesis aimed to build a first model of AA fluxes in the SI of pigs and assess its behaviour in different situations (Chapters 2 & 3).
- The *in vitro* part, aimed to test the use of IPEC-J2 cells cultured on transwells to quantify AA use by intestinal absorptive cells (Chapters 4 & 5).

Thereby, experimental work of this present thesis will be presented in four chapters. Chapter 2 deals with the construction of a conceptual model of AA metabolism in the SI of pig. Choices of the mains elements included are explained and justified, as well as the resulting behaviour of the model. Chapter 3 presents a refined version of this model, which is then used to simulate different nutritional situations and assess their impact on intestinal metabolism of AA. Chapter 4 summaries several trials performed with IPEC-J2 cells and uniformly labelled (^{13}C, ^{15}N) glutamine and lysine to develop an experimental set-up. Chapter 5 presents results from a study which followed the fate of ^{13}C and ^{15}N from uniformly labelled glutamine and lysine incubated with IPEC-J2 cells over time, at two different levels of apical concentration. Cells were cultivated on transwells and labelled AA placed on the apical medium at the start of incubation, then at different time steps, medium (apical and basolateral) as well as cells were harvested and their isotopic composition was measured via EA-IRMS. Finally, chapter 6 consists in a general discussion on methodologies employed, results obtained and future perspectives of research. The four experimental chapters (2, 3, 4, 5) are presented so that they can be consulted separately from the rest of the thesis.

Chapter 2. A conceptual mechanistic model of amino acids fluxes in the small intestine, taking the example of pig

Foreword

We decided to model metabolism of AA in the SI in pig, and its interaction with digestive processes. Construction of such model could help to gain insight on the quantitative importance of AA metabolism in the SI. This model was built based on knowledge available on literature and assumptions we considered reasonable. Obviously, constructing such model could lead to include numerous elements, resulting in a very complex model with the idea of obtaining desired outputs. However, we aimed to make the process of model construction accessible for biologists without background in modelling. The model was built with a principle of simplicity so that the reader can understand how outputs are constructed during simulations. Thus, this chapter focuses on how the model was built, assumptions behind it and resulting behaviour. We believe that this first step of comprehension of the model is necessary before diving into further developments or performing specific simulations.

The present chapter is published in the journal Animal.

Garçon, C. J. J., Le Floc'h, N., Mercier, Y., van Milgen, J. 2025. A conceptual mechanistic model of amino acid fluxes in the small intestine, taking the example of pig. Animal, 19(2), 101414.

Chapter 3. Refining and challenging a mathematical model of amino acids in the small intestine, developed in pig

Foreword

Construction of a mathematical model could provide insights on AA metabolism in the SI and its interaction with digestive processes. The previous chapter introduced the model, its structure, assumptions on which it is based and its general behaviour (Chapter 2). We believed that it was a necessary first step before going further in simulations with the model. In the present chapter, we aimed to present and justify minor refinements of the model's structure. While they do not change the principles and structure of the model, these refinements allow to tackle issues such as AA catabolism in the SI and interactions with non-digestible material in the lumen. The second part of this chapter consists in challenging the model in different nutritional situations. These situations are used to challenge concepts and assumptions on which the model is based, such as the saturation of endogenous secretions in a given segment of SI.

The present chapter is submitted to the journal Animal. The version presented here is the manuscript as it was submitted.

Abstract

A better understanding of the interplay between digestive processes and the metabolism of amino acids in the small intestine (SI) would be valuable for nutritional studies. In a previous study, we proposed a conceptual model of amino acid fluxes in the SI. This model represented the main processes occurring in the SI for a generic AA and represented the SI as a series of segments each retaining the same structure. The model considered AA in different pools, differing the form (i.e., free AA or as protein) and location (luminal or intestinal). The SI is a metabolically very active organ and the model considered intestinal protein turnover. However, for simplicity reasons, certain aspects and nutritional conditions were not yet considered and this included the energy cost of metabolic processes (e.g., protein synthesis and AA transport) and the catabolism of AA (e.g., to provide energy). The initial model considered that endogenous secretions were driven by the presence of intact protein in the lumen, ignoring the presence of other substrates. These aspects were included in the revised model, while maintaining a structure based on a generic AA. The model was then challenged by varying different internal (e.g., different rates of hydrolysis between dietary and endogenous proteins) and external model conditions (diet composition and meal frequency). Addition of catabolism slightly reduced export of AA to blood and forced the SI to take up amino acids from the blood during the nocturnal period. The energy cost of SI metabolism was estimated at 18 mol of ATP per day (i.e., the equivalent of 105 g of glucose), most of which is driven by the cost of protein synthesis (83% of total cost). Increasing the non-digestible content of the diet from 5 to 20% increased more ileal endogenous losses (+ 61 %) than secretions (+ 26 %). This was due to the fact that non-digestible material stimulated endogenous secretions in the distal SI, where secretions are less prone to be hydrolyzed and further absorbed. Increasing meal frequency increased endogenous secretions (+ 33 %), without affecting apparent ileal digestibility, due to more proximal secretions which could be recycled. A decrease in the rate of hydrolysis of endogenous proteins (- 50 %) mainly affected ileal endogenous losses (+ 58 %) and apparent ileal digestibility (-10 %). Overall, the model provides a systemic framework to integrate our current knowledge and views on complex aspects of metabolism that are difficult to address experimentally.

Keywords: protein, *in silico*, digestion, dynamics.

Implications

The small intestine is a crossroad for amino acid metabolism and has an important impact on their appearance in the blood during digestion. In this study, we refined a previously developed model and challenged it in different nutritional situations. Results show that increasing the content of non-digestible material mainly increases endogenous ileal losses rather than secretions. This was due to a prolonged stimulatory effect of non-digestible material in the distal SI on endogenous secretions. This model can be used to better apprehend processes such as endogenous secretions, which cannot be measured *in vivo*.

Introduction

The small intestine (SI) is a crossroad of amino acid (AA) metabolism with AA being provided from and exported to different channels (i.e., lumen or blood). These fluxes can be bidirectional depending on the nutritional status and on the location within the SI. The SI is also a metabolically very active tissue, renewing its protein on a daily basis (Sarri et al., 2024) and representing 20% of the whole body O_2 consumption (Cant et al., 1996). A better understanding of the interplay between digestive processes and intestinal AA metabolism is essential to appreciate the role of the SI in whole animal nutrition. Investigating fluxes of AA in the SI is complicated by its length, the presence of two routes of nutrient supply (lumen and blood) and the rapidity at which processes occur (Leterme et al., 1996). One way to overcome this complexity that is difficult to apprehend experimentally, is to use a modelling approach to study the intestinal metabolism of AA. In addition, a modelling approach provides an alternative to animal experiments.

In a previous article, we developed a conceptual model of AA fluxes in the SI based on existing knowledge (Garçon et al., 2024). It represents the SI as a series of segments, each having the same structure and functioning. The main metabolic processes occurring in the SI were represented for a “generic” AA (i.e., transport, protein synthesis and degradation, exchanges with the blood). The model functioned reasonably well and provided outputs in range with *in vivo* observations. Yet, to focus on the main structural and functional elements of intestinal AA metabolism, several specific aspects of intestinal metabolism were not yet considered.

In the initial model, catabolism of free AA by intestinal cells was ignored, while its importance has been demonstrated, especially for certain non-essential amino acids (NEAA), such as glutamate and glutamine (Wu, 1998). Also, the energy cost of metabolic processes in the SI was also not included, but the portal drained viscera account for 20% of the whole body O_2 consumption, and the SI is expected to be an important contributor to this (Cant et al., 1996). Finally, endogenous secretions in the SI were driven by the presence of dietary protein in the lumen. This was an approximation, because the physical presence of all digesta in the lumen can influence endogenous secretions (Montagne et al., 2003; Stein et al., 2007).

The aim of the present study was to further refine the previously developed model by including additional aspects without modifying its core structure. This includes AA catabolism, the energy expenditure of metabolic processes, and the effect of presence of all digesta components on endogenous secretions. The refined model was further challenged by varying the diet composition, meal frequency and rate of hydrolysis of endogenous proteins.

Material and method

The core model structure

A full description of the initial model is provided by Garçon et al. (2024) but the main features are given here. The model represented the fate of a generic AA in different pools representing the location (i.e., luminal (L) or intestinal (I)), the origin of the AA (i.e., dietary (D) or endogenous (E)), and form (i.e., as protein (P) or as a free AA (F)) of AA (**Fig. 1**). The tubular structure of the SI was represented as a series of functional units called segments. Each segment had the same structure, but operated in a different luminal environment (i.e., quantity of luminal protein and AA). Each segment was composed of seven pools of AA present as luminal dietary protein (*LDP*), luminal dietary free AA (*LDF*), intestinal dietary free AA (*IDF*), intestinal endogenous protein (*IEP*), luminal endogenous protein (*LEP*), luminal endogenous free AA (*LEF*), and intestinal endogenous free AA (*IEF*). Pools were linked by fluxes representing transfer of AA and main metabolic pathways of AA metabolism. The main characteristics of the model were the saturable absorption of luminal AA, homeostasis of intestinal protein and free AA pools (e.g., via exchanges with blood), and endogenous secretions that were driven by the presence of luminal protein. Luminal AA that were not used in any of these processes were

transferred to the next segment. To parameterize the model, data from literature was used and, when not available, plausible values were assumed.

Model development

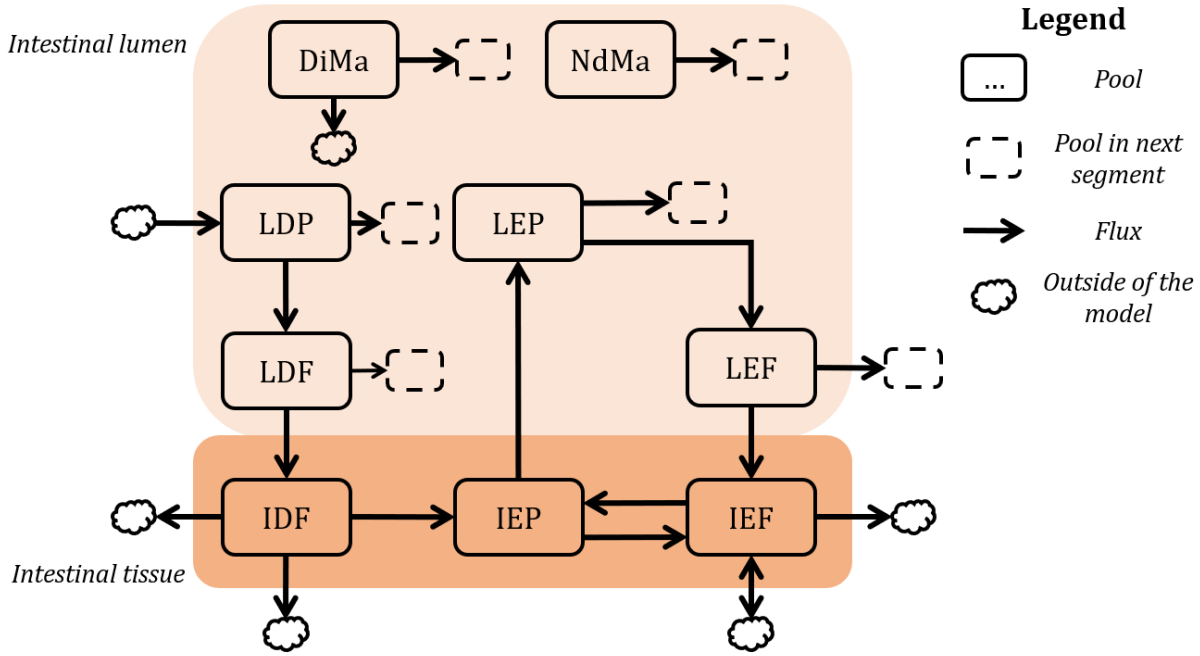


Fig. 1. Scheme of the structure of the model after refinement, for one segment of small intestine. Compartments are represented by squares, fluxes by arrows and clouds represent elements outside boundaries of the model. Dashed squares are compartments in the following segment.

Catabolism of amino acids

Catabolism of certain free AA, especially glutamate and glutamine, is an important feature of intestinal metabolism (Wu, 1998), and was not considered initially. In the refined version of the model, catabolism of free AA was assumed to be proportional to the quantity of free AA present in intestinal tissue (**Fig. 1**). The rate of catabolism (R_{cat}, 0.0015 /min) was set to obtain the equivalent of 10% of dietary AA catabolised over a day.

ATP consumption

It was estimated that the portal drained viscera, which includes the SI, represents approximately 20% of whole body energy consumption (Cant et al., 1996). The transport of ions and nutrients,

synthesis of intestinal proteins as well as their hydrolysis are associated with an energy cost. Based on previous work (Gill et al., 1989; Gerrits et al., 1997), the cost to absorb free AA from the lumen to intestinal tissue was set at 0.33 mol ATP/mol of AA transported. The cost of protein synthesis was set at 4 mol of ATP/mol protein synthetized, and the cost of hydrolysis of intestinal protein to 1 mol of ATP/mol protein hydrolyzed. These changes do not influence the model's behavior but provide additional information.

Digestible and non-digestible material

The first version of the model only considered protein and free AA as material in the lumen. Endogenous secretions in each segment were stimulated by the presence of dietary protein in the lumen (*LDP*). To better represent the interplay between endogenous secretions and the presence of all material in the lumen, the model was modified to include digestible (*DiMa*) and non-digestible material (*NdMa*; **Fig. 1**) other than protein. Digestible material represents all the non-protein material (e.g., starch, lipids…) that can be digested in the SI. The hydrolysis rate of *DiMa* was assumed to be identical to that of protein, but this value can be changed or further differentiated if deemed necessary. Non-digestible material is the fraction of the diet which cannot be hydrolysed in the SI (e.g., fibre). The transit time through the different segments of the SI was assumed to be identical for all fractions that provoke endogenous secretions (i.e., *LDP*, *DiMa*, *NdMa)*. This modification would lead to an increase in total dietary material, so that the parameters of the equation of endogenous secretions (V_{ms}, K_{ms}) had to be adapted. Also, in the initial model, endogenous secretions were close to the maximum value in a considerable part of the SI, and this was considered unrealistic from a biological point of view. Consequently, the parameters of the Michaelis-Menten equation that determine the endogenous secretions (K_{ms}, V_{ms}) were adjusted so that saturation of endogenous secretions only occurred in the proximal SI (i.e., in the first 30 of 200 segments) and that 38 g of endogenous proteins would be lost at the end of the terminal ileum, which is in range with *in vivo* results (Boisen and Moughan, 1996). For the reference situation, we considered a diet containing, in percentage of DM, 16 % protein and 12 % of non-digestible material, the remaining 72 % being digestible material. Intake of protein (Int_{LDP}), digestible (Int_{DiMa}) and non-digestible material (Int_{NdMa}) were adjusted accordingly.

Challenging model behaviour

The revised model was used to investigate the effect of three different challenges on intestinal metabolism. First, the effect of digesta in the intestinal lumen on endogenous secretions. Indigestible substrate will stimulate endogenous secretions throughout the GI tract and this effect was evaluated to carrying out simulations with either a low (5%) or high (20%) non-digestible fraction in the diet.

The meal frequency will determine if the AA required by the intestines come from the diet or the blood, which has become especially important after AA catabolism was included in the model. In the initial model, the daily feed allowance was consumed in five meals of equal size during the day, without feed consumption during the night. In the revised model, this situation was compared with a meal pattern of 12 meals evenly distrusted during a 24 h period. Finally, a decrease (– 50 %) in the rate of hydrolysis of endogenous proteins was investigated so that it differed from the rate of hydrolysis of dietary protein and to assess this effect on endogenous losses.

Model implementation

The model was implemented in the software Python, using version 3.10. The model was built as a series of ordinary differential equations (ODEs) and solved via the solve_ivp function from the package Scipy. One run of the model covered 2 880 minutes (two days), with a step time of 0.5 minute. The 4th order Runge-Kutta method was used to solve the system of ODEs. The first 1 440 min were used to ensure stability of the model outcomes, and only the last 1440 minutes were used in the analysis. The number of intestinal segments (n_{seg}) was set to 200, as a trade-off between run time and realism. The script of the updated version of the model will be available online.

Results

Model refinements

Intestinal catabolism of amino acids and exchanges with blood

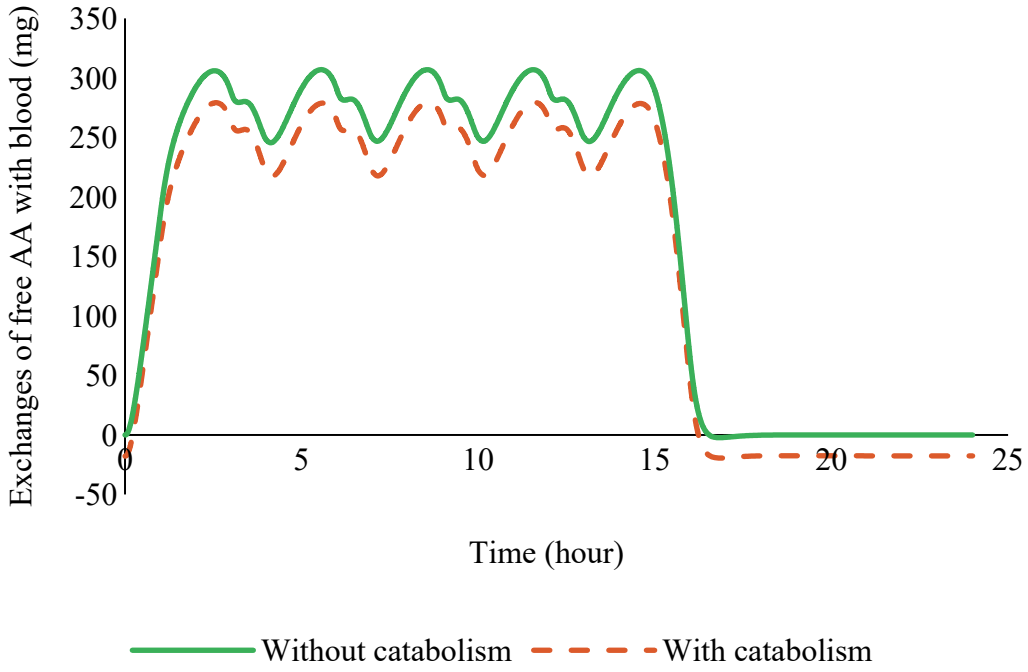


Fig. 2. Comparison of fluxes of exchanges of free AA with blood (*IDF→Ext* + *IEF→Ext*) over time with and without considering AA catabolism. Results are expressed in mg of generic AA and were obtained with 200 segments of small intestine.

The catabolism of AA was set as a constant fraction of the pools of AA in the SI (*IDF, IEF*). Thereby, a peak of AA catabolism was observed during the day in periods of meal, due to the absorption of a large quantity of free AA by the intestinal segments, which fills the pools of free AA. Total amount of free AA catabolised after one simulation represented 34 g, which was equivalent to 11 % of the intake of dietary free AA. The main model outputs affected by addition of catabolism was first-pass metabolism, which was increased from 52 to 56 % of absorbed dietary AA.

Without catabolism of free AA by intestinal tissue, AA were constantly exported to the blood during the day period, when meals are supplied (**Fig. 2**). During the night period, there were no more exchanges of free AA in between the blood and the SI. Addition of catabolism does not change the direction of the flux during the day, as free AA are still exported to the blood,

although exports are slightly reduced. However, during the night – in absence of meals - AA are taken from the blood to the SI, as illustrated by the negative influx in the last hours of simulation. The addition of catabolism of free AA in the SI can be seen as an opening in the system, because it leads to loss of AA within the SI, which needs to be compensated for.

ATP use for intestinal metabolism

Table 1. Energy expenditure as ATP by the small intestine after one simulation of 24 hours, comprising five meals.

Process	ATP (mol)
Total consumption	18
Protein synthesis (*IDF→IEP*, *IEP→IDF*)	15
Luminal free AA absorption (*LDF→IDF*)	1
Intestinal protein hydrolysis (*IEP→IDF*)	2

Simulations were performed in the case of a growing pig of 50 kg of body weight, consuming 2 kg of DM per day, spitted in five meals lasting 1 hour each. **Table 1** represents the ATP use by the SI during 24 hours. Total ATP consumption was 18 mol, mainly due to protein synthesis (15 mol), while absorption of free AA and degradation of intestinal proteins represented a small fraction.

Challenge situations

Effect of non-digestible material in the diet

Table 2. Selected model outputs after one simulation of 24 hours, in a situation of low (5%) or high (20%) non-digestible material in the diet.

	Unit	Low NdMa[1] (5 %)	High NdMa (20 %)	Variation (% of low NdMa)
Endogenous secretions	g/d	146	184	26
Ileal endogenous losses	g/d	38	61	61
First-pass metabolism	% of absorbed	53	58	9
AID[2]	% of intake	83	74	-11

[1]*NdMa* signifies non-digestible material and corresponds to the sum of dietary material which is not degraded by enzymes in the SI (e.g., fibre)

[2]Apparent ileal digestibility

The effect of a variation in non-digestible material content (5 vs 20% *NdMa*) in the diet on model's outputs are shown in **table 2**. Non-digestible material (*NdMa*) has a hydrolysis rate in the intestinal lumen of zero and can be equivalent to the presence of dietary fibre. The level of endogenous secretions increased from 146 to 184 g per day when the *NdMa* content of the diet was increased from 5 to 20%. Endogenous losses were also increased from 38 to 61 g per day. The magnitude of increase in endogenous losses was higher than the increase in secretions (61 vs 26%). The proportion of endogenous secretions which were recycled was reduced from 74 to 67% when *NdMa* content of the diet increased. First-pass metabolism was increased by 9% and apparent ileal digestibility decreased (AID) by 11% with the increase in *NdMa* content of the diet.

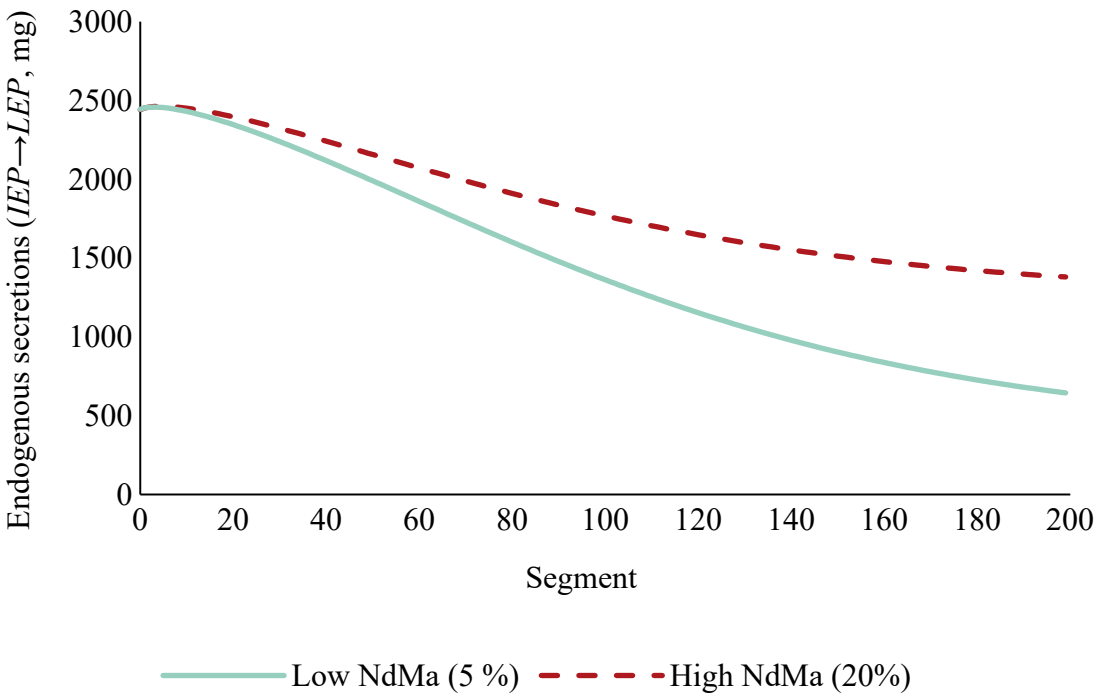


Fig. 3. Comparison of fluxes of endogenous secretions (*IEP→LEP*) over segments of intestine for two non-digestible material (*NdMa*) levels in the diet. Results are expressed in mg of generic AA and were obtained after a simulation of 24 hours with 200 segments of small intestine.

The spatial effect of *NdMa* level on endogenous secretions along the segments of SI is illustrated in **Fig. 3**. The more distal segments are, the higher the difference in endogenous secretions in between low and high *NdMa* diets. This means that, although the presence of *NdMa* overall increases the total level of endogenous secretions (146 vs 184 g), the difference is mainly due to the last two thirds of the SI. This difference occurs because in the high *NdMa* scenario, there is more digesta (i.e., *NdMa*) remaining in the distal SI, stimulating endogenous secretions.

Variation in meal frequency

Table 3. Model outputs in two contrasting situations in term of meal frequency, with a constant quantity per day.

	Unit	Reference meal frequency[1]	High meal frequency	Deviation (% of reference)
Endogenous secretions	g/day	166	220	33
Ileal endogenous losses	g/day	49	55	12
First-pass metabolism	% of absorbed	56	64	14
AID[2]	% of intake	78	77	-2
Intestinal protein synthesis	g/day	401	452	13
Energy consumption	mol/day	18	20	10

[1]In the reference situation, feed intake was separated in two, with a period five meals lasting one hour each, spaced by periods of two hours, followed by 12 hours of fasting. In the high meal frequency situation, feed intake took place continuously over 24 hours, with a meal of one hours every two hours.

[2]Apparent ileal digestibility.

[3]True ileal digestibility.

The effect of increasing meal frequency on ileal endogenous losses was limited (+12%), as reflected by the minor change in AID (Table 3). In the situation of high meal frequency, intestinal protein synthesis was increased by 13% and energy consumption by the SI as ATP by 10%. As indicated in **Table 3**, the high meal frequency situation increased endogenous secretion by 33% compared to reference, from 166 to 200 g/day. In the model, secretions in a segment were driven by the presence of material in the lumen (*LDP, DiMa, NdMa*) but with a maximum rate (V_{ms}). In a situation of more frequent small meals, the intestinal lumen was more frequently filled with digesta which stimulated endogenous secretions. While in the reference situation, a large amount of material was present in the lumen at once, but for a given segment, endogenous secretory capacities were saturated.

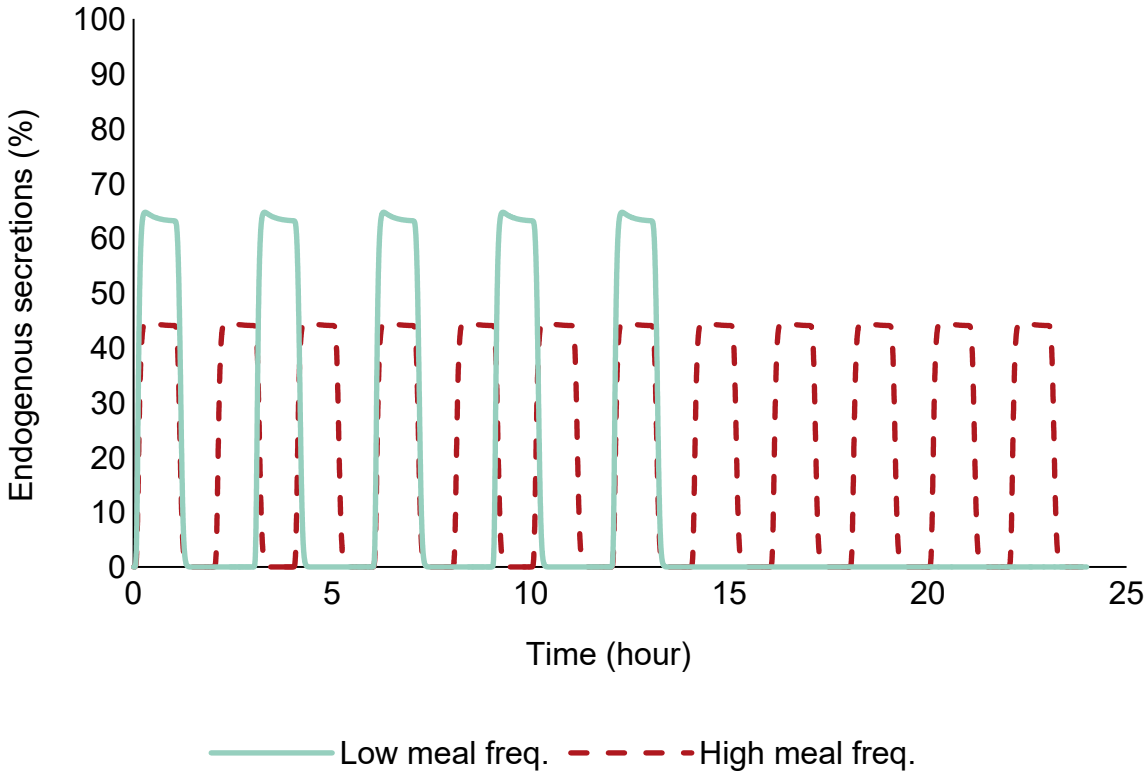


Fig. 4. Endogenous secretions (*IEP→LEP*) over time in segment 10 of the small intestine, for reference and high meal frequency situations. Results are expressed in percentage of maximum secretory capacities of the segment and were obtained with simulations of 24 hours with 200 segments.

The mechanism of saturation of endogenous secretions for the segment 10 of the SI is illustrated in **Fig. 4**. In the period of a meal, the level of endogenous secretions is higher for the reference situation (≈ 65% of max.), than for the high meal frequency situation (≈ 40% of max.) in segment 10. However, there are more feed intake periods in the high meal frequency situations (12), thus more periods of endogenous secretions in the intestinal lumen than the reference situation (5).

Variation of the rate of hydrolysis of luminal endogenous protein

Table 4. Effect of variation in the rate of hydrolysis of endogenous protein (R_{phe}) on model outputs.

	Unit	Variation	
		Reference	- 50 %
Ileal endogenous losses	g/day	49	78
AID[1]	% of intake	78	70

[1]Apparent ileal digestibility.

In the reference situation, rates of hydrolysis of dietary and endogenous proteins in the SI were considered identical. We assessed the effect of a decrease in the rate of hydrolysis of endogenous proteins, because we suspect that endogenous proteins – especially protective ones, such as mucins - are more resistant to hydrolysis than dietary ones. Decreasing the rate of hydrolysis of endogenous proteins increasing ileal endogenous losses, because they are more prone to reach the terminal ileum (**Table 4**). This increase in ileal endogenous losses directly affected AID which was decreased from 78 to 70%.

Discussion

In a previous study, we described a model integrating current knowledge and ideas about the interactions between small intestinal AA metabolism and the digestive processes (Garçon et al., 2024). In the present paper, we further developed this model, by adding AA catabolism, ATP consumption of metabolic processes as well as interactions between dietary fibre and endogenous secretions. Subsequently, the model was challenged in situations of variation of fibre content, meal frequency and rate of hydrolysis of endogenous proteins. These challenges are an opportunity to reflect on concepts on which the model is constructed.

Catabolism of amino acids by the small intestine

Catabolism in the model was implemented in a simple way, as a first order process, at each step time a constant fraction of free AA in intestinal tissue being catabolized. Using data from an experiment conducted in growing pig, fed at a daily feed intake of 2 kg/day with a diet containing 4.5% glutamate and asparagine (de Quelen et al., 2021), we calculated that if both AA are entirely catabolized, a mass of 81 g of AA would be catabolized per day. In the model, catabolism was lower (34 g/day), but in the same order of magnitude. Thereby, the simple parametrization of the model for catabolism allowed to obtain a reasonable output.

However, biological reality is different, as it is known that catabolism is different from an AA to another. Several *in vivo* studies showed that a small fraction of dietary glutamine, glutamate and aspartate reach the blood during digestion (Burrin and Stoll, 2009), but this does not mean that they are entirely catabolised. Numerous studies have shown the importance of NEAA such as glutamine, glutamate and aspartate as energy sources of intestinal cells (Wu, 1998). Yet, the extent to which they are catabolized remains unclear. For EAA, it seems that catabolism is very limited, but for example branched chained amino acids (BCAA) are deaminated in intestinal cells (Chen et al., 2009); which is a form of partial catabolism. Some AA such as alanine or proline are also synthetized from other AA by the SI, thus catabolism does not mean a loss of AA *per se*. The current version of the model does not allow to distinguish in between different AA. Yet, accounting for the specific case of glutamine, as an illustration, would not require to change the structure of the model. Rate of catabolism could be adjusted to reach the equivalent of 100 % of digestible dietary glutamine catabolized per meal and model behavior could be evaluated.

Energy use by the small intestine

Direct *in vivo* energy consumption by the SI is estimated by measuring the O_2 consumption by the portal drained viscera (PDV) in the pig, which includes – but is not limited to - O_2 consumption by the SI. The PDV represents only 5% of body weight but up to 25% of oxygen consumption in pig (Yen et al., 1989), due to its very high metabolic activity. The model estimated that the daily energy expenditure was equivalent to 18 mol of ATP (i.e., 0.71 mol of ATP/hour). By using data of O_2 consumption from two *in vivo* studies using pigs (Yen et al., 1989; Yen and Nienaber, 1993), we obtained a rate of ATP consumption per hour by the PDV

between 0.85 to 1.24 mol of ATP/hour, during fasting and fed period respectively. Our estimate is in a similar range, but lower, knowing that it comprises only SI ATP consumption, while *in vivo* estimate comprises the whole PDV. Several studies estimated the *in vitro* rate of O_2 consumption by small intestinal cells in culture. Using the data from Nyachoti et al. (2000), a rate of ATP consumption of 0.32 mol of ATP/hour can be obtained. Active transport systems have been pinpointed as the main source of energy consumption by the gastrointestinal tract of ruminant, closely followed by protein synthesis (McBride and Kelly, 1990). It is not the case in this model, in which protein synthesis was by far the main driver for ATP consumption, representing 83 % of total intestinal ATP consumption. Active transport includes the cost of transport of other nutrients such as glucose, and ion balance, processes which were ignored in this model, explaining the low contribution of transport to total energy consumption.

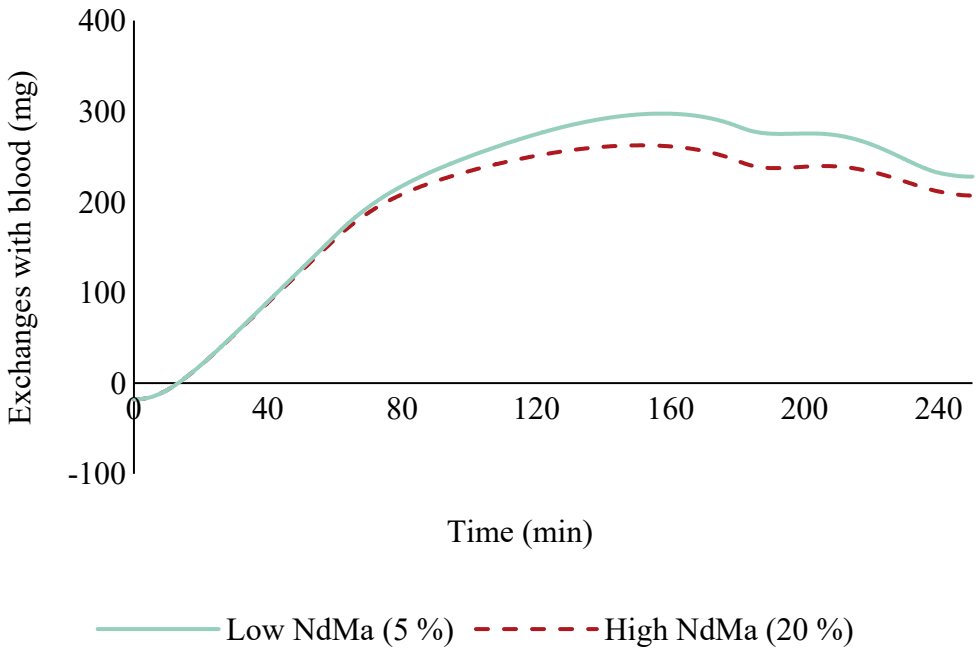
Sources of energy for the small intestine

Amino acids such as glutamine, glutamate and asparagine are considered as the main energy sources for intestinal cells (Wu, 1998). Glutamine and glutamate have been identified as preferential substrate for energy production, compared to glucose using isolated pigs enterocytes *in vitro* (He and Wu, 2022). However, the question remains whether dietary glutamine, glutamate and asparagine are - in addition to be preferential substrates, quantitatively enough to supply energy to enterocytes. In the model, daily energy requirements of the SI were of 18 mol per day. Potential ATP production from dietary glutamate (being the sum of glutamate and glutamine) and asparagine was estimated using diets from the experiment of de Quelen et al. (2021) and considering 22 mol of ATP produced per mol of glutamine catabolised and 14.75 per mol for arginine (van Milgen, 2024). A potential production of 13 mol of ATP per day from dietary glutamate and arginine was estimated, considering that both AA were entirely catabolised by the SI. This means that together, glutamate, glutamine and arginine could potentially fulfil around 70% of energy requirements of the SI. This result suggests that AA can play a quantitatively important role in the energy supply to the SI. This estimate of 13 mol of ATP per day is probably an upper limit, because the catabolism of AA may be partial, part of the carbon from glutamate and glutamine can be retained in alanine and released by the SI.

Concepts of specific and basal endogenous losses

In the model, endogenous secretions differ from endogenous losses that are typically determined at the end of the terminal ileum in *in vivo* studies. This is because part of the endogenous secretions will be hydrolyzed in the lumen and the resulting AA can be absorbed before arriving at the terminal ileum. These secretions thus contribute to intestinal AA metabolism without contributing to the endogenous ileal losses. Endogenous ileal losses can be determined experimentally and are assumed to be a function of the characteristics of the diet. Endogenous ileal losses are composed of basal endogenous losses, which are proportional to a quantity of material ingested (in DMI) and specific endogenous losses, which are dependent on characteristics of the diet such as the presence of fiber or antinutritional factors (Stein et al., 2007). Specific endogenous losses are often expressed in proportion of dietary AA content of the meal, due to the potential stimulatory effect of this AA rich material on losses (Stein et al., 2007). No distinction is currently made between the different components of endogenous secretions (e.g., sloughed cells, mucus, enzymes), or for the fact that they occur along the SI and over a period of time of several hours (Boisen and Moughan, 1996). These processes were represented in the model in a simple way. The nature of endogenous secretions, the factors provoking their secretion, and the hydrolysis in the SI may be considered in future refinements of the model.

Fig. 5. Fluxes of total exchanges of free AA by the small intestine with blood (*IDF→Ext* + *IEF→Ext*) over time, in situation of low (5%) or high (20%) *NdMa* content in the diet. Results are expressed in mg of generic AA and were obtained after a simulation of 1440 minutes with 200 segments of small intestine.



Interactions between low digestible material and intestinal metabolism

In the present model, non-digestible material had a hydrolysis rate of zero, but any material with a low digestibility (i.e., resistant starch) could behave in a similar way in the intestinal lumen. A low digestible material would by its prolonged presence in the lumen stimulate endogenous secretions in the distal lumen. Thus, the kinetic of hydrolysis of material is more important than final hydrolysis itself and was the most important element describing the interactions between endogenous losses and digesta in the lumen in the model.

Dietary fibre plays an important role in stimulating endogenous secretions in the SI (Montagne et al., 2003). Presence of dietary fibre in the intestinal lumen corresponds presence of material with a hydrolysis rate of zero, which is the case of non-digestible material *(NdMa)* in this model. Using pigs of 15 kgs of BW, Schulze et al. (1994) found an increase from 34 to 48 g of endogenous ileal losses in proteins, when purified NDF content of the diet varied from 0 to 20 % of DMI. This figure is in a close range of results obtained here, losses increasing from 28 to 61 g, with 5 to 20% non-digestible material in the diet. Increasing the non-digestible material content in the diet had a more pronounced effect on endogenous losses (+ 61%) than on secretions (+ 26%). This is due to the fact that, as illustrated in **Fig. 3**, addition of non-digestible material to the diet leads to presence of digesta all along the digestive tract, thereby stimulating

endogenous secretions. These secretions are then less prone to be hydrolyzed and will appear in the terminal ileum as endogenous losses.

Increasing non-digestible material content of the diet also leads to a slight increase in first-pass metabolism, from 53 to 58% of absorbed dietary AA. The observed increase in first-pass metabolism is due to the increase in endogenous secretions observed in the high *NdMa* situation, which drives intestinal protein synthesis. As illustrated in **Fig. 5**, non-digestible material reduced exports of AA to the blood after a meal, especially after the meal is terminated (after 60 min). This decrease is due to the fact that endogenous secretions are prolonged over time, due to the presence of undigested material (*NdMa*) remaining in the lumen after the meal, while digestible material quickly disappeared. Non-digestible material has thus a double consequence, prolonging secretions over time, as well as increasing the contribution of distal SI to endogenous secretions.

Meal frequency

Continuous feeding led to an increase in endogenous secretions compared to the reference situation (220 vs 166 g/day). This can be explained by the fact that the secretions are proportional to the quantity of material (*LDP, DiMa, NdM*) in the lumen as well as saturable properties of endogenous secretions. In fact, the high meal frequency scenario leads to a longer period during which material is present in the lumen over the entire simulation, stimulating endogenous secretions. In the high meal frequency scenario, there was less material present in the lumen per meal, thus less stimulation of endogenous secretions. However, as illustrated in **Fig. 4**, due to the saturable property of endogenous secretions, this decrease in the magnitude of secretions per meal (≈ 3.9 vs 2.7 mg) was compensated by the higher number of meals (5 vs 12).

In comparison to the reference situation, ileal endogenous losses only minorly increased (12%) in the high meal frequency situation. This is explained by the fact that in a high meal frequency situation, most of digestive processes occur in the proximal SI, because of the lesser quantity coming per meal. These endogenous proteins secreted in the proximal SI can then be degraded during the rest of their transit through the SI. In a study in which meal frequency was increased from two to eight meals per day in pig, no effect was found on AID, although a slight numerical

reduction. It is coherent with results which obtained in the model, were AID only decreased from 78 to 77 % with the increased meal frequency (Le Naou et al., 2014).

Saturation of endogenous secretions in the model

The diet represents a large quantity of nutrients, which drives endogenous secretions in the digestive tract. Splitting the diet into meals as well as progressive stomach emptying reduces the amount of digesta entering the SI at the time. In the model, amount of digesta in the SI is further divided by the organization as a series of functional segments along which digesta transits. In the first segments of the model, secretions were implemented with a saturation so that they are limiting compared to the amount of digesta entering. Each segments secrets proteins for its protections (e.g., mucins) as well as to contribute to digestion (e.g., enzymes). In the model, one segment cannot secret endogenous protein for the whole SI and has limited capacities represented by the saturation in endogenous protein secretions. In this study, we did not consider pancreatic juice and bile, which also enter the first segments of the SI but are secreted and are active along the whole length of the SI. These elements could be further considered when refining the model. If further consideration was to be given on endogenous secretions, there rate of hydrolysis could also be investigated. In fact, it could be expected that protective secretions such as mucins have a lower rate of hydrolysis.

In summary, we refined and challenged an existing model of intestinal metabolism of AA. This extended model included aspects of AA catabolism, dietary fibre and energy costs of metabolic processes. The increase of dietary fibre increased more importantly endogenous secretions than losses. Increasing meal frequency did not affect digestibility, but increases endogenous secretions, which can in turn increase the metabolic cost of intestinal metabolism. Such simulations help to obtain a different spotlight on results previously obtained *in vivo* and suggests new paths of investigation to better understand digestive physiology.

Ethics approval

Not applicable.

Data and model availability statement

The code of the model on Python will be available online.

Declaration of generative AI and AI-assisted technologies in the writing process

None.

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J. van Milgen: supervision, project administration, conceptualization, writing - review & editing.

Declaration of interest

None.

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Appendix 1. Parameters of the model, with their name, corresponding signification, unit and value.

Parameter	Signification	Unit	Value
Int_{LDP}	Intake of protein	mg/min	1 067
Int_{DiMa}	Intake of digestible material	mg/min	4802
Int_{NdMa}	Intake of non-digestible material	mg/min	800
n_{seg}	Number of intestinal segments		200
R_{phd}	Rate of luminal dietary protein hydrolysis	/min	0.018
R_{phe}	Rate of luminal endogenous protein hydrolysis	/min	0.018
R_{phm}	Rate of digestible material hydrolysis	/min	0.018
MRT	Mean retention time	min	180
K_{ma}	Affinity constant for absorption	mg	40
V_{ma}	Maximum rate of absorption	mg/min	0.0065
R_{ps}	Rate of protein synthesis	/min	0.06
R_{pd}	Rate of intestinal protein hydrolysis	/min	0.000694
T_{IP}	Target of AA^2 in intestinal protein	mg	$240\ 000/n_{seg}$
T_{IF}	Target level of intestinal free AA	mg	$12\ 000/n_{seg}$
R_{fb}	Rate of exchange with blood	/min	0.06
V_{ms}	Maximum rate of endogenous secretion	mg/min	0.00175
K_{ms}	Affinity constant of endogenous secretion	mg	20
R_{cat}	Rate of intestinal AA catabolism	/min	0.0015
ATP_{tra}	ATP cost of apical AA absorption	mol of ATP	0.33
ATP_{syn}	ATP cost of intestinal protein synthesis	mol of ATP	4
ATP_{hyd}	ATP cost of intestinal protein hydrolysis	mol of ATP	1

Chapter 4. Setting-up an *in vitro* method to study the fate of amino acids incubated with IPEC-J2 cells on transwells

Foreword

In this thesis, we aimed to set-up an *in vitro* method to investigate the fate of AA incubated with intestinal cells. We sought that such method could be used to provide data to further parametrise the model presented in the last two chapters of this thesis. To our knowledge, the use of intestinal absorptive cells cultivated as a polarised layer to study AA metabolism is scarce in literature, with the exception of one study (Sakai et al., 2020). Thereby, they were many unknown to perform this experiment, ranging from the choice of the appropriate medium for cell culture, the timespan at which processes occur, or the quantity of material which could be harvested to perform measurements. The series of trials presented in this chapter was performed to set-up the method, both technically and scientifically. Results are presented here in a step-by-step manner, to reflect the process of progressive construction of the method over time. Discussion of the results is focused on methodological aspect. Further reflection on the scientific interest of this method is presented in the following chapter. We aim to valorise results obtained here in a short note.

Introduction

The idea of using cultivated cells to study intestinal AA metabolism is not new, as illustrated by the work of Watford et al. (1979) to investigate glutamate metabolism in isolated chicken and rat intestinal mucosal cells. Isolated intestinal cells were then widely used in the 1990s, especially by Wu and collaborators to identify the presence of metabolic pathways and assess substrate preferences by intestinal cells sampled on animals (mainly piglets) just after slaughter (Wu, 1998). Most of these studies used cells cultivated in tubes or wells and incubated for a short period of time (≈ 30 min) in a buffer media with the addition of an AA of interest.

In the SI, intestinal mucosa cells form a layer of polarised cells bound to each other and digestive processes occur in a dynamic manner in term of time (Leterme et al., 1996). These properties were not reflected in experiments using intestinal cells collected and isolated after animal slaughtering. Conversely, cell lines, such as Caco-2 or IPEC-J2 can be cultivated on transwells, forming a layer over a porous membrane, with an apical and basolateral side, as in the intestinal epithelium. This polarised layer of intestinal cells could provide in interesting model of AA absorption by the intestinal epithelium. In addition, cell lines have the advantage of having well defined culture conditions (Faria et al., 2024) and provide more replicable results compared to the use of cells directly sampled on animals. As an illustration, Sakai et al. (2020) studied the fate of glutamate labelled with stable isotopes incubated in the apical medium with Caco-2 cells cultivated on transwells.

The aim of this study was to use *in vitro* cultured cells to assess their potentiality to study AA fluxes across intestinal epithelial cells. IPEC-J2 cells cultivated on transwells were used, because they have the advantage of being non-transformed small intestinal mucosal cells originating from the jejunum of unsuckled pigs (Berschneider, 1989). Fate of AA in intestinal cells can be very different from an AA to another, with some NEAA such as glutamine being intensely catabolised to be used as energy source (Wu, 1998). Conversely, EAA like lysine are presumably not catabolised by intestinal cells (Chen et al., 2009). Thereby, lysine is expected to be incorporated in proteins or to cross the cell layer to be released in the basolateral side. Thus, it was decided to compare the fate of glutamine with lysine, the latter being rarely used by intestinal cells. These two AA also have the advantage to have a very similar molecular weight (146 g/mol), which can influence their diffusive capacities over a cell layer. As

processes occur in the SI in a dynamic manner, we performer a kinetic to follow the fate of glutamine and lysine over time. To follow the kinetic of use of glutamine and lysine incubated with IPEC-J2 cells, stable isotopes labelling was used (^{13}C, ^{15}N).

The present chapter summarises several trials using IPEC-J2 cells cultivated on transwells, to assess its interest as a model of fluxes of AA in the intestinal epithelium. This series of trials was conducted in a step-by-step manner, the following trial being constructed based on the results of the previous one. Material and method is given in details for the first trial, then for the followings, only the differences with the previous one are given.

Trial n°1 – Glutamine incubated over two hours

Material and method

Study design

The aim of this first trial was to assess the practical feasibility of the method, with a short kinetic and using uniformly labelled glutamine. In fact, only a low amount of DM can be harvested per transwell (<10 mg) and the possibility to measure enrichment over time remained unclear. After 11 days of culture, cells on transwells were incubated with uniformly labelled glutamine in the apical medium. A short kinetic over three time points (15, 60 and 120 min) was performed, with four replicates (i.e., transwells) per time point. At each time-point time, plates were taken out of the incubator, medium (apical and basolateral) was harvested as well as the cell layer. These samples were then analysed for their isotopic composition via EA-IRMS, to assess which fraction of ^{13}C and ^{15}N from the labelled AA was retained in the apical medium, incorporated in the cell layer or passed in the basolateral medium (**Figure 1**).

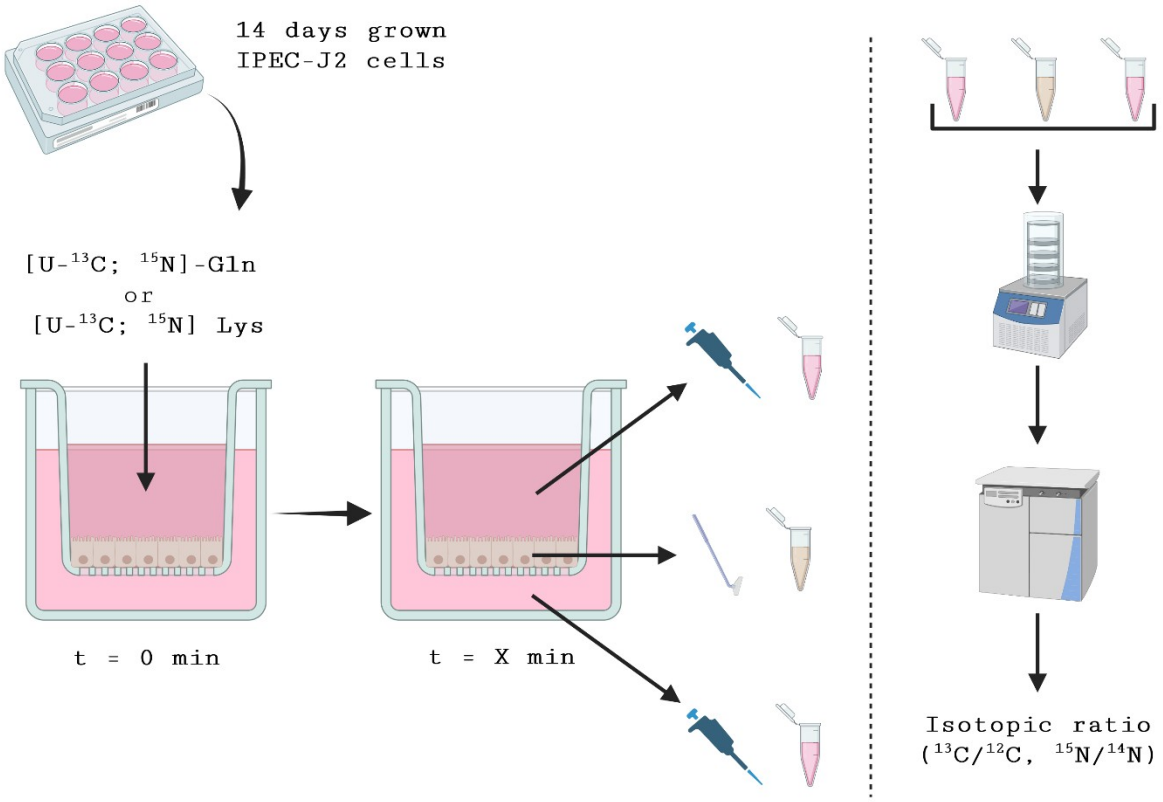

Figure 1. Simplified scheme of the general experimental procedure, as well as sample analysis, for this series of trials.

Culture of IPEC-J2 cells

IPEC-J2 cells were grown on 75 cm^2 flasks (Thermo Scientific, catalogue no. 156800) in Dulbecco's Modified Eagle Medium/Ham's F-12 containing L-Glutamine and Sodium bicarbonate (DMEM/Ham's F-12, Gibco catalogue no. 11320-033) supplemented with 10 % porcine serum (PS, Gibco, catalogue no. 26250-084) filtered, decomplemented, and containing 1 % penicillin-streptomycin (Gibco catalogue no. 15070- 063). Flasks were placed in a humidified (95 %) incubator at 37 °C with 5 % CO_2 and culture medium was replaced every two to three days. When cells reached confluence in one flask, medium was removed and cell layer was rinsed before being incubated for 10 min in 5 mL of Trypsin-EDTA 0.25 % (Gibco, catalogue no. 25200-072). After incubation, cells were centrifugated at 600 G, at 20°C for 5 minutes and supernatant was discarded. After centrifugation, cells forming a pellet were suspended in culture medium and could potentially be seeded in new flasks, with a density of 10^6 cells per flask, to maintain a stock of cells further available.

In this experiment, cells from passage three were used and seeded into transwells polyester membrane inserts (0.4 µm pore size, 1.1 cm^2 surface area, Starstedt, catalogue no. 83.3931.040), in 12-well plates at a density of 1×10^5 cells per cm^2 (Starstedt, catalogue no. 83.3921.005). Cells were grown on transwells during 11 days, depending on the trial in a humidified atmosphere (95 %) incubator at 37 °C under 5 % CO_2. Media was replaced every two to three days, with 1 mL in the apical chamber and 2 mL in the basolateral one. Transepithelial electrical resistance (TEER) was measured every few days with Epithelial Voltohmmeter (EVOM3, Friedberg, Germany). TEER values, in $\Omega \times cm^2$, were calculated as follows:

$$TEER = (R - R0) \times A$$

where R is the measured resistance (Ω), R0 is the blank resistance (transwell without cells), and A is the transwell effective surface (1.1 cm^2). After confirming epithelial cell differentiation by a TEER value (> 300 Ω cm2), transwells were used for experiments. This culture protocol was partly developed with the help of Perruchot et al. (2025). Results for the TEER are presented for all the trials combined in one graph, at the end of results from trial n°3.

Stable isotope experiment

Plates were taken out of the incubator, transwells were placed in new plates according to their time-point and initial medium was removed. The basolateral chamber was filled with 2 mL of Roswell Park Memorial Institute medium 1640 (RPMI, Gibco, catalogue no. 21870) supplemented with 0.51 mmol/L L-glutamine (Gibo, catalogue no. 25030-024) and containing 1 % penicillin-streptomycin. At time 0 minutes, in the apical chamber, 800 µL of the initial medium was removed and replaced with the same volume of RPMI 1640 containing 1 % penicillin-streptomycin as well as 2.05 mmol/L of L-Glutamine of which 5 % was [U-^{13}C; ^{15}N]-Glutamine (Sigma Aldrich, catalogue no. 607983) and 45 % was [U-^{13}C]-Glutamine (Sigma Aldrich, catalogue no. 605166). One additional transwell was filled with 800 µL of RPMI 1640 containing 1 % penicillin-streptomycin as well as 2.05 mmol/L of L-Glutamine, without label (blank). Plates were then incubated as determined by the protocol at 37°C, with humidified atmosphere and 5 % CO_2. At each time point, corresponding plate was taken out from the incubator and placed on ice. A volume of 800 µL of apical and basolateral medium was sampled and placed in 2 mL Eppendorf tubes, which were directly placed in liquid nitrogen. Remaining medium in apical chamber was discarded and apical chamber was rinsed with a volume of

500 µL of Hans balance salt solution (HBSS, Thermo Fisher, catalogue no. 14025092). Then, cells were gently removed from the membrane of the transwell using a cell scraper (Starlab, catalogue no. cc7600-0220). Transwells were then rinsed twice with 300 µL of molecular biology grade water (Corning, catalogue no. 46-000-Cl) and liquid containing cells was pipetted in a tube which was then place in cold nitrogen. **Table 1** summarises the main methodological characteristics of each trial. All samples were then stored at - 80°C before analyses.

Table 1. Main characteristics of each of the three methodological trials.

	Trial n°1	Trial n°2	Trial n°3
AA studied	Glutamine	Glutamine & Lysine	Lysine
Culture medium during isotopic experiment	RPMI1640	RPMI1640 & DMEM/F12	RPMI1640
Cells passage	3	5	2
Labelled AA	[U-^{13}C; ^{15}N]-Glutamine, [U-^{13}C]-Glutamine	[U-^{13}C; ^{15}N]-Glutamine, [U-^{13}C]-Glutamine, [U-^{13}C; ^{15}N]-Lysine	[U-^{13}C; ^{15}N]-Lysine
Apical concentration[1]	Gln: 2.05 mmol/L	Gln, Lys: 2.05 mmol/L	Lys: 2.05 mmol/L
Basolateral concentration[2]	Gln: 0.51 mmol/L	Gln, Lys: 0 mmol/L	Lys: 0.22 mmol/L
Time points	15, 60, 120 min	0, 30, 240 min	10, 20, 30, 45, 60, 90, 120, 240
Replicates/time	4	3	1
Pre-incubation	No	No	Yes (1 hour)
Days of culture	11	14	14
Rinsing of apical chamber	1 x 0.5 mL HBSS	1 x 0.5 mL HBSS	2 x 0.5 mL HBSS

[1]Sum of labelled plus unlabelled lysine and glutamine.

[2]Only unlabelled AA was present in basolateral chamber at time zero.

Samples preparation

Frozen samples were freeze dried for 48 to 72 hours. Samples were weighted to obtain the dry matter content. Samples containing media (apical or basolateral) were grinded for 30 seconds at 1300 rotations per minutes with two 2 mm inox beads and 1 – 2 mg of ground power was placed in and then placed in thin capsules for isotopic analysis. For cell samples, due to the limited amount of material available (<1 mg), the totality of the sample was placed in thin capsules.

Sample analysis via EA-IRMS

Elemental content and the isotope composition were measured on culture medium or cells with a CHONS Elemental Analyzer (EA Vario PYRO cube; Elementar® Elementar, Villeurbanne, France) coupled to an isotope ratio mass spectrometer (IRMS; Isoprime precision, Elementar®). The possible offsets of raw IRMS values were corrected using both international IEAE (Vienna) and home-made standards (a sucrose–methionine–glycine mixture, referred to as SMG, both cross-calibrated against caffeine IAEA-600 and Ag2S IAEA-S-1). Note that the linearity of IRMS response was checked throughout to be less than 0.02 ‰ n_A-1, and furthermore, any possible size effect between samples was very small.

Calculations

Isotopic composition was expressed in the conventional delta notation, for ^{13}C or ^{15}N:

$$\text{Delta } ^{13}C \text{ or } ^{15}N \ (\Delta, \text{ in } ‰) = [(R_{sample} - R_{standard})/R_{standard}\ ] \text{ x } 1000$$

where R is the heavy/light isotope ratio, and standard material are international reference materials (V-PDB, air N_2 for ^{13}C, ^{15}N respectively). Delta in a given sample was then corrected for the natural abundance of ^{13}C or ^{15}N s as followed:

$$\text{Delta } ^{13}C \text{ or } ^{15}N \ (\Delta, \text{ in } ‰)_{\text{ corrected}} = \text{delta}_{sample} - \text{delta}_{blank}$$

Where delta_{sample} is the delta measured in the sample and delta_{blank} is the delta measured in corresponding non-enriched sampled (apical medium, basolateral medium or cells). Thus, any values above zero signify a net enrichment.

Mass of ^{13}C or ^{15}N in a sample was calculated by first expressing percentage of ^{13}C or ^{15}N in sample, as follows:

$$p\ (\%) = (R_{sample})/(R_{sample}+1)\ x\ 100$$

Then, mass of ^{13}C or ^{15}N present in one sample was calculated as:

$$Mass\ (g) = m_s\ x\ (p/100/M)$$

Where m_s is the mass of C or N in a given sample (g) and M is the molar mass of ^{13}C or ^{15}N (g/mol).

Results & discussion

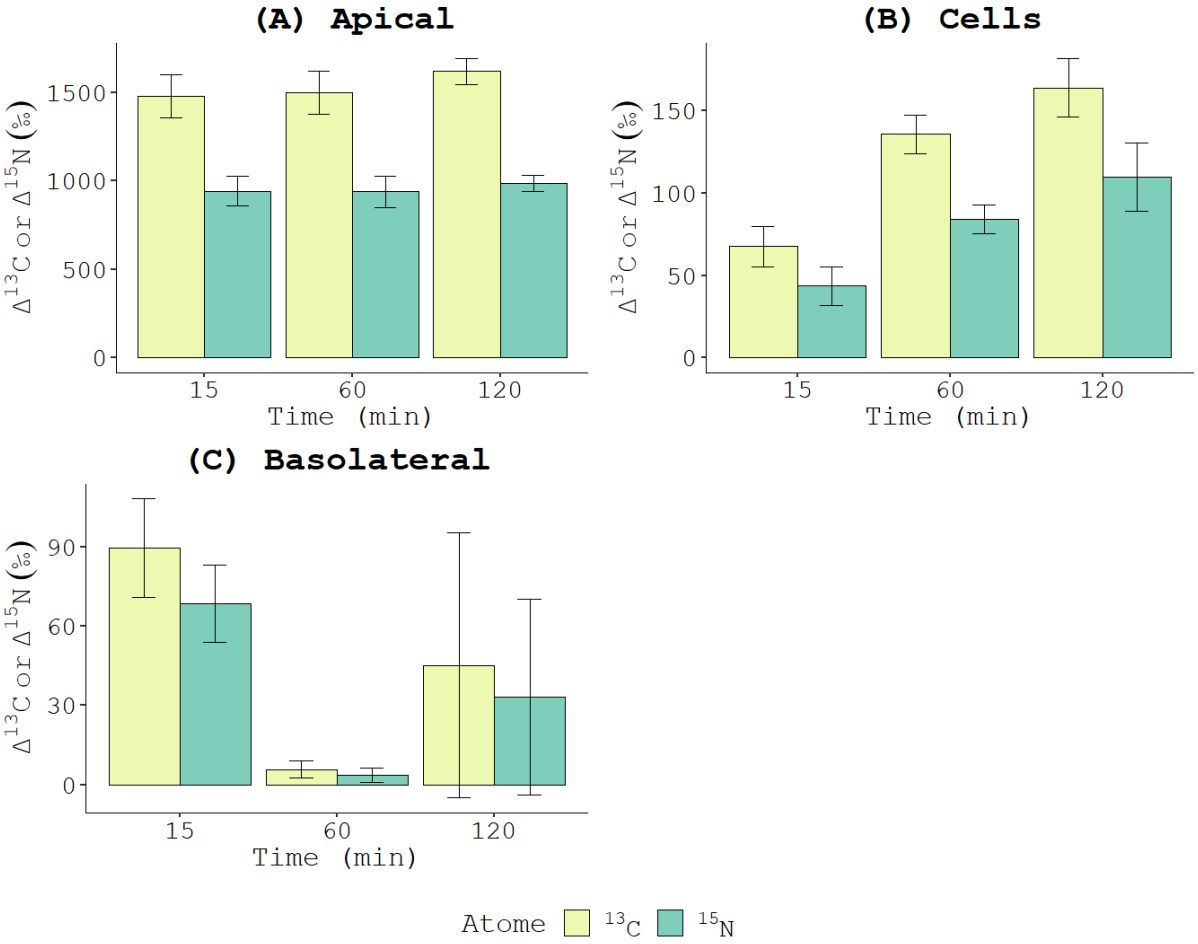

Figure 2. Isotopic composition in delta (Δ, ‰) for ^{13}C (yellow) or ^{15}N (green) in the apical, basolateral medium or IPEC-J2 cells, at three different time point (15, 60, 120 min). Data shown are means ± standard deviation (n = 4). Values above zero signifies a net enrichment compared to blank samples.

Measurement of delta is based on a ratio (*R*) in between a light and naturally rare isotope (e.g., ^{13}C) and a heavier more abundant one (e.g.,^{12}C). The value of delta is the expression of the deviation of this ratio *R* toward the ratio of an international standard (e.g., Pee Dee Belemnite for C, $^{13}C/^{12}C = 0.011$). In the present study, samples were enriched in heavy isotopes (i.e., ^{13}C or ^{15}N) by the presence of [U-^{13}C; ^{15}N]-Glutamine. Thus, when interpreting delta values, it must be kept in mind that it is the relative enrichment in ^{13}C or ^{15}N over ^{12}C or ^{14}N respectively, that is measured.

In the apical medium, enrichment remained stable over time, for both ^{13}C and ^{15}N (**Figure 2A**) showing that the isotopic composition remained stable in the apical medium over the period of 2 hours. It seemed that glutamine present in the apical medium, which is partly composed of [U-^{13}C; ^{15}N]-Glutamine and [U-^{13}C]-Glutamine was not absorbed to larger extent than other nutrients containing C and N present in the medium. In fact, otherwise a variation in $\Delta^{13}C$ or $\Delta^{15}N$ over time would be observed, due to a change in the isotopic composition of the medium. It was expected that IPEC-J2 cells, which are intestinal cells, would absorb a large amount of glutamine to use it as energy substrate (Wu, 1998). It seems that it was not the case in the timespan of this experiment, or that enrichment in ^{13}C and ^{15}N was too high in the apical medium to detect it.

Measuring enrichment in cells was a challenge due to the limited amount of dry material harvested per transwell (≈ 0.7 mg), yet it was possible to obtain detectable variations in isotopic composition in cell samples (**Figure 2B**). A progressive increase in $\Delta^{13}C$ and $\Delta^{15}N$ over the three time-points was observed. This result shows that cells absorb ^{13}C and ^{15}N from labelled glutamine, presumably in the form of glutamine, originating from the apical medium. Delta in cells was much lower than in apical medium (≈ 10 times less), and cells represented only >1 mg of DM, while there was ≈ 10 mg in apical medium. Thus, the fraction of ^{13}C and ^{15}N from apical medium absorbed in the cell layer seemed relatively low.

In the basolateral medium, results in $\Delta^{13}C$ and $\Delta^{15}N$ were more variable over time (**Figure 2C**). Delta was already reaching 90 and 70 ‰ for ^{13}C and ^{15}N respectively, after 15 minutes, which is above other time points. This result suggests that an important amount of ^{13}C and ^{15}N from labelled glutamine had crossed the cell layer after 15 minutes. However, enrichment after 60 minutes was much lower than after 15 min ($\Delta^{13}C$ and $\Delta^{15}N < 5$ ‰). and increased again after

120 minutes. Enrichment in the basolateral medium at 120 minutes was highly variable in between replicates (SD = 50 and 37 for $\Delta^{13}C$ and $\Delta^{15}N$ respectively). Altogether, these results show that in the basolateral medium, kinetic of appearance of ^{13}C and ^{15}N from glutamine is unclear. It could be speculated that integrity of cell layers might differ from one transwell to another, explaining the high variations observed in between time point and replicates. We speculated that individual variation in the integrity of cell layers could be reflected in measurement of TEER. However, TEER at day 9 and delta in basolateral medium were not correlated, for both ^{13}C and ^{15}N (p-value>0.05). The ≈ 20-fold differences in $\Delta^{13}C$ and $\Delta^{15}N$ observed in between both mediums (apical and basolateral) indicates that overall, a limited fraction of heavy isotopes from glutamine present in the apical medium crossed the cell layer to reach basolateral medium.

Results from this trial confirmed that cells absorbed part of the labelled glutamine present in the apical medium, and that it was detectable in the cells. However, in the basolateral medium results were less clear due to high variation in $\Delta^{13}C$ and $\Delta^{15}N$ among time-points and replicates. Overall, results from cells and basolateral medium suggest that only a small fraction of labelled glutamine disappeared from the apical medium during the period of 2 hours. It was suspected that the culture medium used in this experiment (RPMI 1640) was too rich in nutrients, and especially AA which can compete in between each other's for transporters (Bröer and Fairweather, 2018). This competition might have limited the uptake of the labelled glutamine we introduced in the apical medium. Thus, we sought to use a nutrient poor medium (HBSS) in the next trial to push IPEC-J2 cells to uptake labelled AA from the apical medium. In this trial, glutamine was present in basolateral medium (0.51 mmol/L) and might have been used by intestinal cells; competing with apical use. We thus decided for trial n°2 to have no glutamine in the basolateral medium, to make sure that cells rely only on apical supply. Finally, during of the incubation was increased from 2 to 4 hours to assess if more variation could be detected over a longer time period.

Trial n°2 – Comparison of culture media

Material and method

Study design

This experiment aimed to improve absorption by IPEC-J2 cells, by increasing concentration gradient in between apical and basolateral chambers as well as using a nutrient poor medium. We used HBSS as a nutrient poor medium, as it contains only salts and glucose. A second objective of this experiment was to compare the behaviours of glutamine and lysine. They were three treatments in this trial, first one being with glutamine in RPMI 1640 as in the first trial, but without glutamine in the basolateral medium (GlnRPMI). The second treatment consisted of glutamine in HBSS medium (GlnHBSS), and the last one was with lysine in HBSS medium (LysHBSS). A kinetic over three time points, 1, 30 and 240 min was performed, with three replicates (transwells) per time point. For this experiment, plates were used after 14 days of culture in 12 wells plates, instead of 11 days (**Table 1**), to harmonise culture conditions within laboratory (Perruchot et al., 2025). Only the differences with the previous trials are presented below.

Stable isotopes experiment

At day 14, the basolateral chamber was filled with 2 mL of either RPMI 1640 without L-glutamine and containing 1 % penicillin-streptomycin (GlnRPMI) or HBSS containing 1 % penicillin-streptomycin (GlnHBSS, LysHBSS). On the apical chamber, 800 µL of initial medium was removed and replaced with the same volume of medium, according to one of the three treatments: GlnRPMI, GlnHBSS or LysHBSS. For GlnRPMI treatment, apical medium contained RPMI 1640 with 1 % penicillin-streptomycin as well as 2.05 mmol/L of L-Glutamine of which 5 % was [U-^{13}C; ^{15}N]-Glutamine (Sigma Aldrich, catalog no. 607983) and 45 % was [U-^{13}C]-Glutamine (Sigma Aldrich, catalogue no. 605166). For the GlnHBSS treatment, apical medium contained HBSS with 1 % penicillin-streptomycin as well as 2.05 mmol/L of L-Glutamine, of which 1.0 % was [U-^{13}C; ^{15}N]-Glutamine and 9.6 % was [U-^{13}C]-Glutamine. Finally, for the LysHBSS treatment, apical medium contained HBSS with 1 % penicillin-streptomycin as well as 2.05 mmol/L of L-Lysine Hydrochloride (Thermo Scientific, catalogue no. J62099.22) of which 1.0 % was [U-^{13}C; ^{15}N]-Lysine Hydrochloride (Sigma Aldrich,

catalogue no. 608041). LysHBSS medium was also supplemented with 2.05 mmol/L of L-Glutamine, in the apical chamber. Incubation of the plates was then performed in the same conditions as the previous trial, as well as sampling procedure. To increase the amount of harvested cells, the porous membranes were rinsed twice with 500 mL biology grade molecular water, instead of 300 mL in trial n°1.

Results and discussion

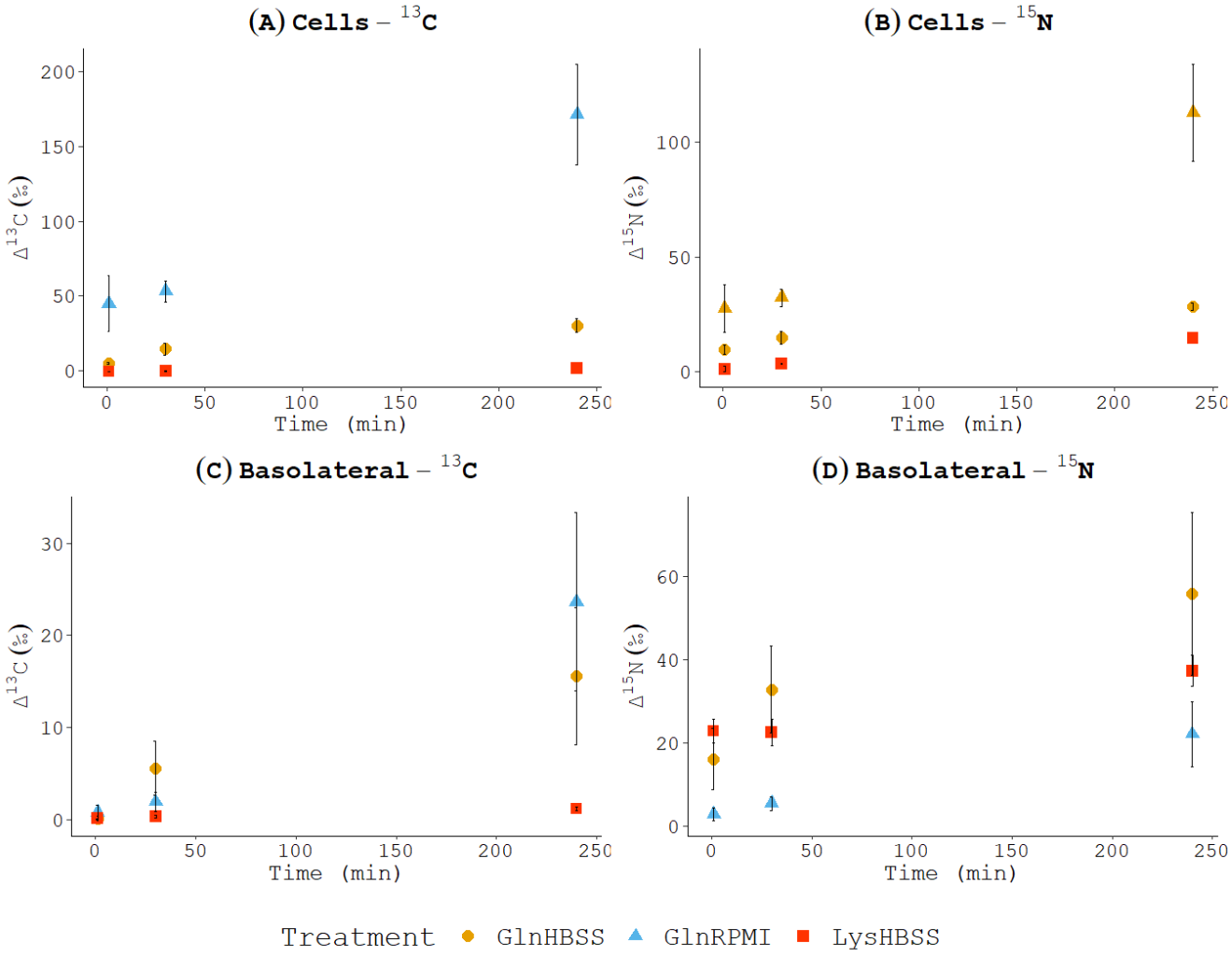

Figure 3. Isotopic composition in delta (Δ, ‰) for ^{13}C or ^{15}N in the basolateral medium or IPEC-J2 cells, at three different time point (1, 30, 240 min), with three different culture conditions (GlnRPMI, GlnHBSS, LysHBSS). Data shown are means ± standard deviation (n = 3). Values above zero signifies a net enrichment compared to blank.

As in the previous trial, enrichment in the apical medium remained stable over time, for each treatment (data not shown). In cells, $\Delta^{13}C$ and $\Delta^{15}N$ increased over time, regardless of the treatment, indicating absorption by the cells (**Figure 3A, B**). This increase in $\Delta^{13}C$ and $\Delta^{15}N$ was the highest for GlnRPMI, indicating that in RPMI culture medium, more ^{13}C and ^{15}N from glutamine was absorbed by cell layers than with HBSS as culture medium. Absorption of ^{13}C and ^{15}N from lysine in the cell layer was very limited, either due to low absorption of lysine itself or the fact that cells were incubated in HBSS medium. Delta in cells was already above zero for both GlnRPMI and GlnHBSS at time 1 min, which means that already part of the ^{13}C and ^{15}N from glutamine was absorbed by the cell layers. It is also possible that procedure of rinsing of the apical chamber was not thorough enough to remove all trace of labelled AA before harvesting the cells.

In the basolateral medium, there seemed to be a steady increase in delta over time for each treatment, which indicates that ^{13}C and ^{15}N from labelled AA passed in the basolateral medium over time (**Figure 3C, D**). Exception can be made for $\Delta^{13}C$, with the LysHBSS treatment, which remained very close to zero. While $\Delta^{15}N$ in basolateral medium for LysHBSS treatment was already around 20 ‰ after 1 min of incubation and remained stable over time. We observed a similar situation with the GlnHBSS treatment, for which $\Delta^{13}C$ was around 1 ‰ at time 1 min while it was reaching 16 ‰ for $\Delta^{15}N$. These differences in delta between C and N could be due to the fact that HBSS medium does not contain ^{14}N, while it contains ^{12}C from glucose (5 mmol/L). Thus, even a tiny fraction of ^{15}N from glutamine or lysine passing in apical medium already leads to a high increase in delta in the basolateral medium. In this trial, direct comparison in between medium (HBSS or RPMI 1640) is complicated by the fact that their composition in term of C and N differs to a large extent. Overall, there seemed to be a progressive passage in the basolateral medium, bar lysine for which it seemed that the kinetic over time was flat.

In summary, this trial showed absorption of ^{13}C and ^{15}N from labelled glutamine and lysine in the cell layer, as well as passage in the basolateral medium. From data obtained in the cells, it seemed that using a medium poor in nutrients (HBSS) reduced absorption of ^{13}C and ^{15}N from glutamine, thus probably reducing the absorption of glutamine itself. Lysine seemed to be less absorbed by the intestinal cells than glutamine, although further experiments with a nutrient rich medium (RPMI 1640) would be required.

Trial n°3 – Lysine in a nutrient riche medium

Material and method

Study design

The aim of this trial was to evaluate the kinetic of C and N from lysine, incubated in RPMI 1640 medium. Besides, during the first trial, an important variation in $\Delta^{13}C$ and $\Delta^{15}N$ in basolateral medium was observed. It was suspected that it was due to the abrupt change of medium as performed in previous trials at time 0 minutes impaired integrity of the cell layer. Thus, in this third trial a pre-incubation step of one hour in fresh medium was added before the addition of a volume of solution containing labelled lysine. Finally, this trial was an opportunity to perform a kinetic including more time points, over the same time period (4 hours). One kinetic of measurement was performed, with one replicate per time point at times: 10, 20, 30, 45, 60, 90, 120 and 240 min. Again, only differences in the protocol with the previous trial are indicated below. In parallel, an assay was performed to evaluate the permeability of cell layers during the incubation period, using lucifer yellow-CH di-potassium (LY).

Stable isotope experiment

At day 14, the basolateral chamber was filled with 2 mL of either RMLI 1640 without L-glutamine and containing 1 % penicillin-streptomycin. On the apical chamber, 800 µL of initial medium was removed and replaced with 750 µL RPMI 1640 medium containing 1 % penicillin-streptomycin and 2.05 mmol/L of L-Glutamine. Plates were then placed in the incubator for one hour. After one hour, 50 µL of a solution with a concentration of 36.88 mmol/L of lysine Hydrochloride containing 6 % [U-^{13}C; ^{15}N]-Lysine Hydrochloride was added in the apical chamber of each transwell, to obtain a final concentration of 2.05 mmol/L of lysine Hydrochloride. Incubation and sampling procedure were the same as in the previous trial, except that rinsing of the apical chamber before scrapping of the cells was done twice with 500 mL HBSS, instead of only once. This was done in order to make sure that all the ^{13}C and ^{15}N from the apical medium was removed before retrieving the cells.

Permeability assay

IPEC-J2 cells were seeded at a density of 1×10^5 cells/mL in transwells placed on twelve wells plates. Cells were grown as in previous experiments, until day 14 in DMEM/F-12 medium

containing L-Glutamine and Sodium bicarbonate, supplemented with 10% porcine serum filtered and decomplemented, and containing 1 % penicillin-streptomycin. At day 14, TEER was measured and entirety of basolateral medium was removed as well as 800 µL of apical medium. Then, apical chamber was rinsed once with 0.8 mL HBSS. Then, DMEM/F12 with no phenol red (Thermo Fisher, catalog no. 21041025) was added, with 2 mL in the basolateral chamber and 0.7 mL in the apical one. Plates were then placed in the incubator again at 37°C, with humidified atmosphere and 5 % CO_2 for 3 hours. After, 100 µL of a solution containing HBSS as well as 200 µmol/L of lucifer yellow-CH di-potassium (LY, Sigma-Aldrich, catalog no. L0144-25mg) was added in the apical chamber and cells were incubated again. Steps involving LY were performed with a limited amount of light in the laboratory. At time 15, 60, 120 and 240 minutes, 150 µL was sampled twice from the basolateral chamber and placed in 96 well plate black. Basolateral medium was then refilled with 300 µL of HBSS before plates were incubated again. Fluorescence was then measured at an excitation wavelength of 428 nm and an emission of 540 nm with a micro-plate reader (Varioskan Lux, ThermoFisher, Villebon-sur-Yvette, France). The LY concentration, in µmol/L, was determined from a standard curve (0 to 200 µmol/L).

Results and discussion

Delta ^{13}C and ^{15}N

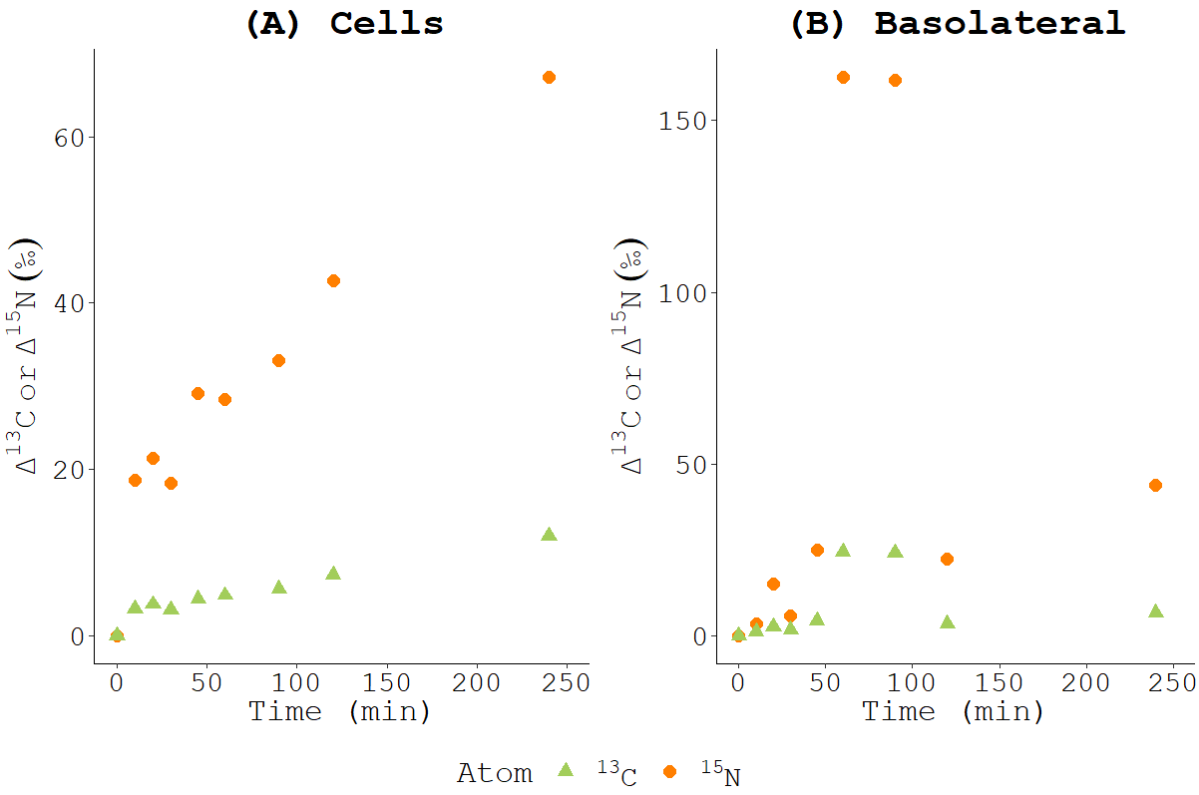

Figure 4. Isotopic composition in delta (Δ, ‰) in ^{13}C (green triangles) or ^{15}N (orange dots) in the apical, basolateral medium or IPEC-J2 cells, over a kinetic of 240 minutes. Values above zero signifies a net enrichment compared to blank.

As observed in the previous trials, $\Delta^{13}C$ and $\Delta^{15}N$ remained stable over time in the apical medium (data not shown). In cells, delta increased linearly over time, indicating that cells absorbed and incorporated ^{13}C and ^{15}N from lysine during the incubation period (**Figure 4A**). A detectable enrichment was already measured after 10 minutes in cells, which suggests that cells rapidly absorbed lysine from the apical medium.

In the basolateral medium, as for trial n°1, no clear trend can be drawn from the kinetic over time (**Figure 4B**). However, basolateral medium is enriched (delta above zero), which indicates that ^{13}C and ^{15}N from lysine passed from the apical medium. At time 60 and 90 minutes in basolateral medium, $\Delta^{13}C$ and $\Delta^{15}N$ is higher than any other time points. It could suggest that

part of both markers had already passed the cell layer, possibly due to an impaired permeability. In fact, by looking at enrichment in the apical medium at these time point (same transwells), it seemed that it was slightly lower than other time points (data not shown). Even by removing these time points (60 and 90 minutes), no clear kinetic of evolution of $\Delta^{13}C$ and $\Delta^{15}N$ in basolateral medium could be observed over time. In this trial, we have added a pre-incubation step in which medium was replaced one hour before the addition of a small dose of AA containing liquid (50 µL). In fact, we suspected medium replacement to alter the integrity of the cell layers and allow fast passage of heavy isotopes (^{13}C, ^{15}N) in the basolateral medium. In the present study, it did not seem that adding the pre-incubation step improved the quality of the kinetic of evolution of $\Delta^{13}C$ and $\Delta^{15}N$ in basolateral medium.

Results from this last trial showed that cells incorporated ^{13}C and ^{15}N from lysine and that part of these atoms also ends up in the basolateral medium, without a well-defined kinetic. To go further in the analysis, we expressed results from this trial in mass of ^{13}C or ^{15}N, instead of delta. In fact, expressing results in mass allows to more easily compare results, regardless of the matrix and to get a quantification of the fate of ^{13}C and ^{15}N from labelled lysine.

Mass of ^{13}C or ^{15}N

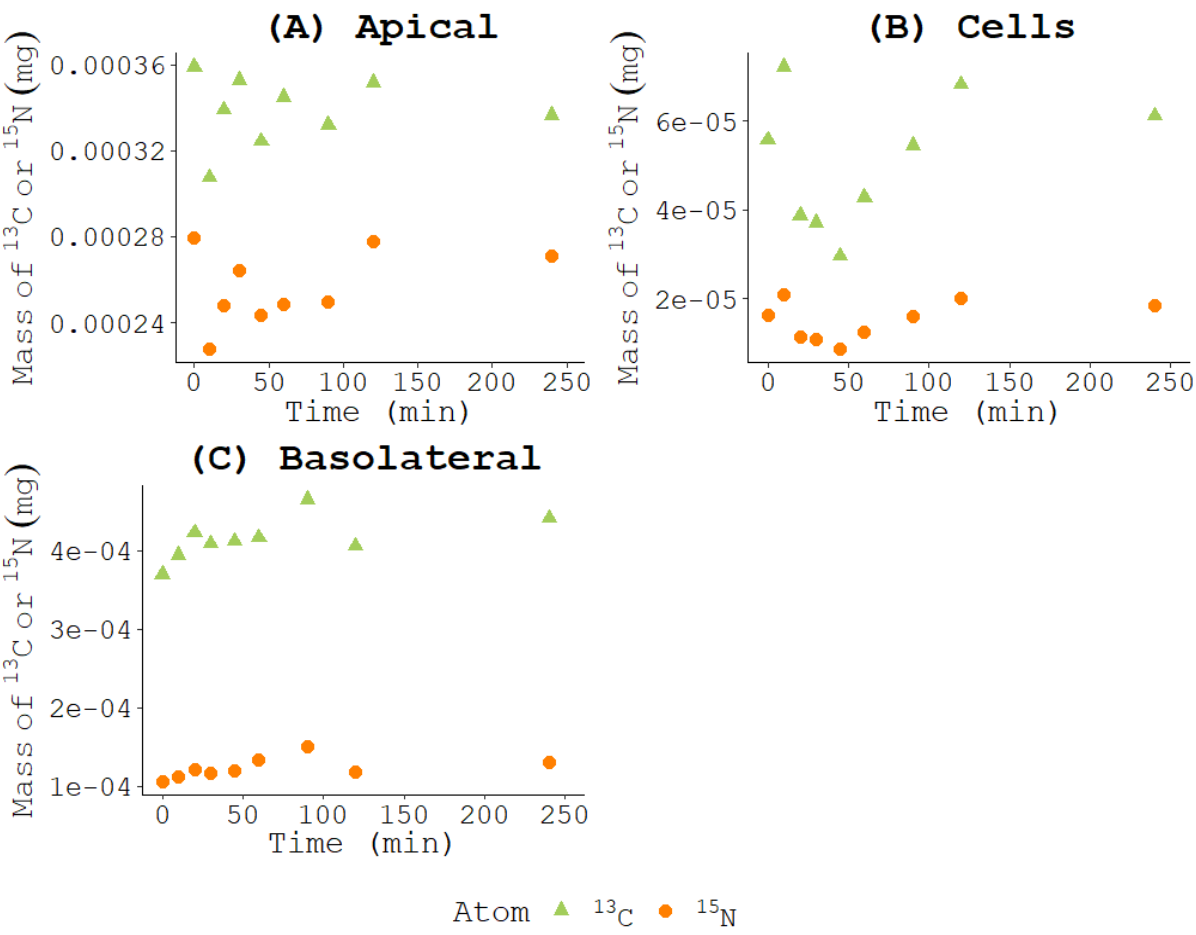

Figure 5. Mass in mg of ^{13}C (green triangles) or ^{15}N (orange dots) in the apical, basolateral medium or IPEC-J2 cells, over a kinetic of 240 minutes.

Using the isotopic ratio (*R),* the mass of samples, and their composition in C and N, the mass of ^{13}C and ^{15}N for each sample was calculated. This was done to obtain a quantitative estimate of the fraction of ^{13}C or ^{15}N present in each matrix, per time point. In the apical medium, quantity of ^{13}C and ^{15}N remained relatively close to its initial value, although with some variations (**Figure 5A**). If only a small fraction of labelled glutamine is absorbed as previous results suggested, it might not be detectable by expressing results in mg. In cells, it was difficult to observe any trend over time, although a linear increase can be observed in between time 45 and 120 minutes, for both ^{13}C and ^{15}N (**Figure 5B**). This is probably due to the fact that quantity of C and N in the cell layer varied from one sample to another, which added noise into our

calculations. In basolateral medium, it seemed that the quantity of ^{13}C and ^{15}N increased slightly over time, although there is no clear kinetic (**Figure 5C**). For each of the matrixes, variations in the mass of ^{13}C or ^{15}N were minor over time. These calculations require a precise weighting to obtain the exact dry weight of the sample. As the quantity of material (≈ 0.5 – 10 mg) was very small compared to the weight of the tube used (≈ 1.2 g), it is possible that it impaired precision of the measurement. It can also be speculated that variation in quantity of cells (thus C and N) per transwell varied and lead to some noise in estimates of ^{13}C and ^{15}N recovered. In fact, calculation of the quantity of ^{13}C and ^{15}N is based on the isotopic ratio, which is obviously influenced by the initial quantity of light isotopes, namely ^{12}C or ^{14}N. We tried to reduce this variability per transwell by expressing quantity of ^{13}C or ^{15}N per mg of C or N in cells, with the idea that it might control variability in between cell layer. However, results obtain this way did not show any clear trend neither (data not shown).

Mass of cells sampled

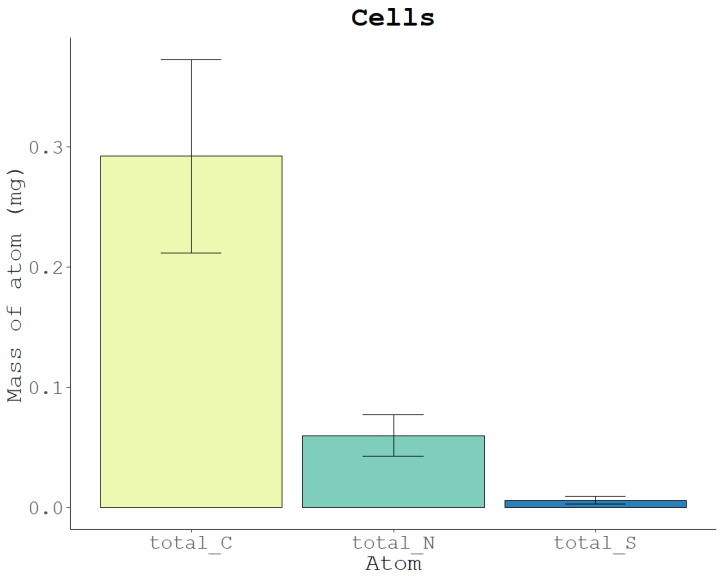

Figure 6. Quantity of C, N and S (in mg) in IPEC-J2 cells for each time points. Data shown are means ± standard deviations (n = 8).

Samples of harvested cells weighted on average 0.7 mg and were mainly composed of C (≈ 0.3 mg), followed by N (≈ 0.05 mg) and small amount of S (≈ 0.005 mg), **Figure 6**). There was variability in the content of C and N in between transwells, which probably impacted the

calculation of the quantity of ^{13}C or ^{15}N recovered. Variability in the composition of C and N is both due the variation in percentage of C and N in samples as well as the quantity of cells harvested. Whether this variation in quantity of cells harvested was due to the quantity of cells present in a transwell or due to the harvesting process itself remains unclear.

Permeability assay

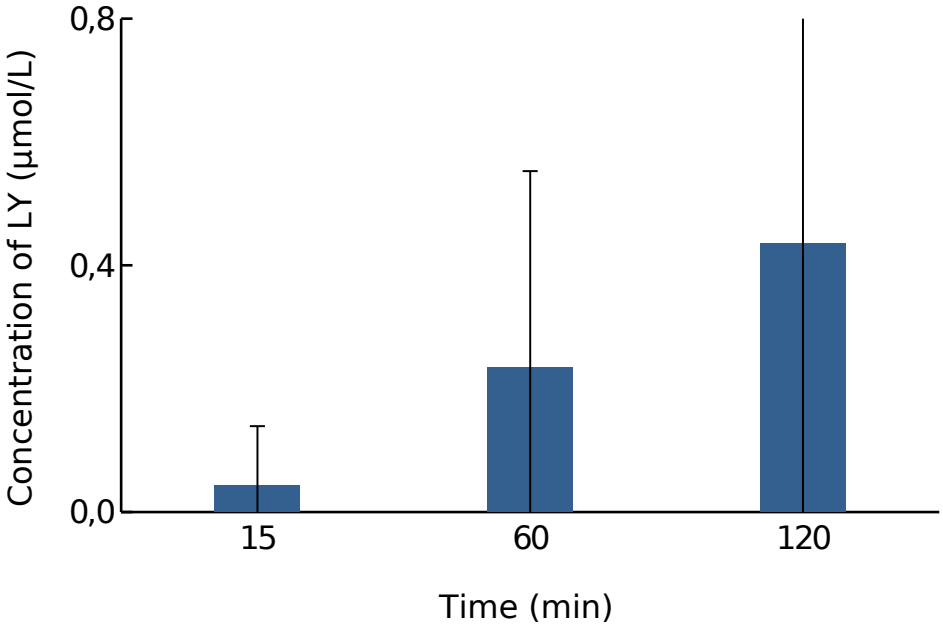

Figure 7. Concentration of luciferase yellow (LY) in the basolateral medium, at three different time points. IPEC-J2 cells were incubated on transwsells with a concentration of 200 µmol/L in the apical medium at time zero, in DMEM/F12 with no phenol red.

Permeability was assessed by incubating IPEC-J2 cells with LY at a concentration of 200 µmol/L in the apical medium. Over the period of 120 minutes, concentration of LY increased until reaching 0.4 µmol/L (**Figure 7**). This result indicates that passage of LY over the incubation period of 2 hours was very limited. Thereby, we can conclude that the culture conditions used in stables isotopes experiments did not impair the integrity of the cell layer. LY is however a heavier molecule than an AA (522 vs 146 g/mol for glutamine and lysine). Thus, it is still possible that part of the AA diffused through the layer in some transwells, resulting in rapid passage of lysine and glutamine.

Transepithelial electrical resistance (TEER)

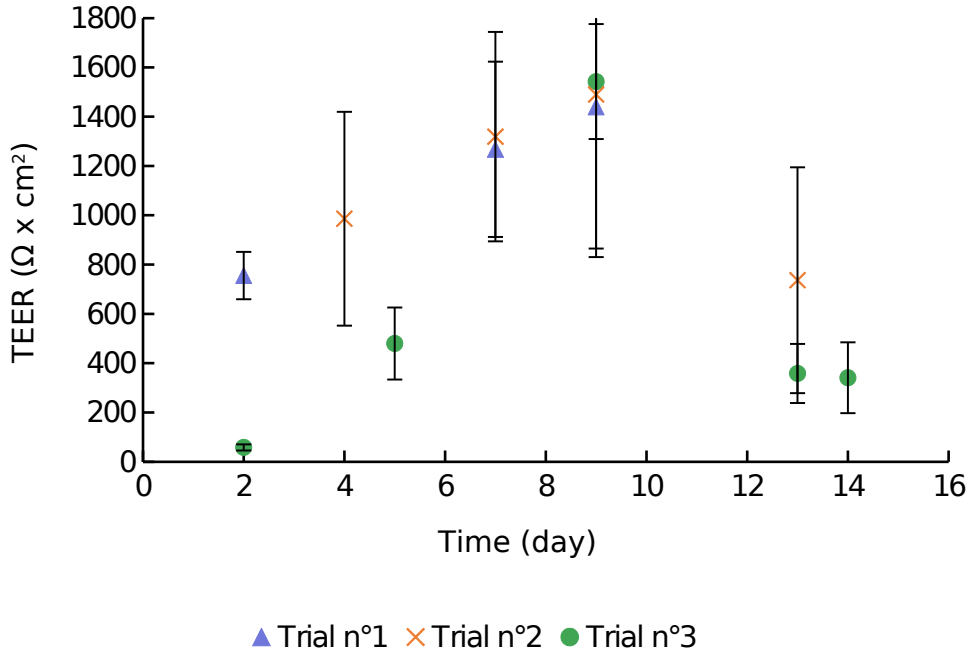

Figure 8. Evolution of transepithelial electrical resistance (TEER) over time, during each trial. IPEC-J2 cells were cultivated on transwells in DMEM/F12 medium containing 10 % porcine serum and 1 % penicillin-streptomycin.

TEER was measured at several time points during each trial, as an indicator of the integrity of cell layers. In all trials, TEER increased until day 9 and reached its peak at around 1500 Ω/cm^2 (**Figure 8**). At days 13 and 14, values from trial n°2 and 3 showed a decrease and a stabilisation of the TEER, around 500 Ω/cm^2. In trials n°1 and 2, variability of TEER was high at most time points, while for trial n°3, it remained in close range in between transwells. As an illustration, at day 13, for trial n°2, TEER ranged from 0 to 1771 Ω /cm^2, depending on the transwell. Values of TEER were difficult to interpret due to their high variation, however, they were used as an indicator to discard transwells when too low (< 300 Ω/cm^2).

Conclusion from trials and perspectives

This series of experiment was performed in order to assess the feasibility of a method using cultured cells (IPEC-J2) on transwells to follow the kinetic of use of ^{13}C and ^{15}N from two AA which are differentially metabolised by intestinal cells. To this end, we compared the kinetic of ^{13}C and ^{15}N from glutamine and lysine over time, incubated with IPEC-J2 cells, with successive improvements in the protocol.

Our results show that cell absorbed a detectable amount of ^{13}C and ^{15}N from both glutamine and lysine over time. It seemed that more AA were incorporated into the cell layer using a nutrient rich medium (RPMI 1640) than a nutrient poor one (HBSS). We also observed passage of ^{13}C and ^{15}N in the basolateral medium, although with more variability in the results over time, potentially due to diffusion through the cell layer. However, looking at LY experiment, it seems that cell layers maintained their integrity during the experiments. Expressing results in mass (mg) of ^{13}C or ^{15}N did not prove to be successful, due to the very limited amount of materiel available (0.5- 10 mg), which probably impaired the weighting process. These trials confirmed the feasibility of the experiment and provided culture conditions in which a larger trial can be performed, to compare the kinetic of glutamine and lysine incubated with IPEC-J2 cells.

Chapter 5. Fate of carbon and nitrogen from glutamine and lysine incubated with IPEC-J2 cells cultivated on transwells

Foreword

The present study was performed after that the method was developed as explained in the previous chapter (Chapter 4). The aim of this study was to assess the potential use of IPEC-J2 cells cultivate on transwells and incubated with labelled AA (^{13}C, ^{15}N) to study the metabolic fate of AA in intestinal cells. To this end, we followed the kinetic of ^{13}C and ^{15}N from lysine and glutamine incubated with IPEC-J2 cells at two different apical concentrations. The aim was to observe if differences in the behaviour of atoms from AA which are differentially metabolised by intestinal cells could be observed in this experimental set-up. And if varying apical concentration of AA had an effect on the saturation of absorption by IEC-J2 cells. This trial was performed with the idea that *in vitro* culture of intestinal cells could be a way forward to obtain data about fate of AA in the SI, which cannot be obtained *in vivo*. This knowledge would be valuable to complement the mathematical modelling approach presented in chapters 2 and 3. Results from this trial have recently been obtained thus, further analysis is required to draw clear conclusions. Nevertheless, these results are presented and some preliminary ideas are discussed.

Introduction

Protein digestion and AA absorption in the SI results the appearance of AA in the portal blood. It is well known that the profile of AA appearing in the blood during the digestion and available for post-absorptive metabolism differs from that of dietary proteins. Indeed, the SI extensively metabolises AA, which are used for protein synthesis or catabolised for energy production. The quantification of AA metabolism was performed several decades ago using catheterised animals and tracers administration (Bertolo et al., 1998; Bos et al., 2003). In addition to methodological limits, such approaches using live animals, mainly pigs, are based on invasive protocols that should be limited due to ethical concerns.

In a previous study, a mechanistic model of AA fluxes in the SI was constructed, based on current knowledge on AA metabolism (Garçon et al., 2024). This model was built to get insights on the quantitative importance of intestinal metabolism and its interactions with digestive processes. Yet, certain data to parametrise the model were lacking and are a difficult to obtain using *in vivo* studies. A way forward to obtain these data and complement the model would be the use of *in vitro* cultured intestinal cells.

Intestinal cells cultivated *in vitro* have been used to study aspects of intestinal AA metabolism (Wu, 1998), such as substrate preference for energy production (He and Wu, 2022), presence of metabolic pathways (Wu, 1995) or enzymes activity (Chen et al., 2009). Yet, in these studies, intestinal cells were incubated during a short period of time (≈ 30 min) and in static conditions. These experimental conditions do not reflect the reality of the SI, in which digestion is a dynamic process, with processes occurring rapidly (Leterme et al., 1996). In addition, these cells were not organised as a polarised layer, while it is an important feature of epithelial intestinal cells.

The IPEC-J2 cell line is derived from unsuckled piglet jejunum and has been used in research since several decades (Vergauwen, 2015). Compared to Caco-2 cells, a cancer cell line from colon, IPEC-J2 line has the advantage of being a non-transformed cell line originating from the SI. Its use has mainly been restricted to the investigation of the barrier function intestinal epithelium under different conditions (Tretola et al., 2021; Perruchot et al., 2025). These cells can be grown on transwells, forming a polarised layer, exhibiting properties of intestinal epithelial cells such as microvilli in their apical surface - presence of tight junctions proteins

and glycocalyx bound mucins (Schierack et al., 2006). These features indicate that IPEC-J2 cells could potentially be used as a model to study AA metabolism by the SI.

The aim of the present study was to investigate the potential of IPEC-J2 cells grown on transwells to study AA metabolism by intestinal cells over time. To assess the relevance of the cell model, fate of uniformly labelled glutamine and lysine (^{13}C, ^{15}N) incubated in the apical medium of IPC-J2 cells was investigated. Glutamine was used because it is an important energy source for enterocytes (He and Wu, 2022). It is expected that catabolism of glutamine would result in differences in behaviour in between ^{13}C and ^{15}N from glutamine during the incubation period. Besides, lysine is an essential AA that should not be catabolised by intestinal cells (Chen et al., 2009); thus it was expected that it would be released intact in the basolateral side – without differences in the behaviour of ^{13}C and ^{15}N. In addition, two apical concentrations for the given AA were tested. It was expected that if AA are taken up by active transporters in the apical membrane of cells (Bröer and Fairweather, 2018), a saturation of absorptive capacities of cells would be observed at high concentration.

Material and method

Experimental design

Four treatments, depending on the AA studied (glutamine or lysine) and the apical concentration of the AA of interest (high or low) were compared. Depending on the AA studied, a fraction (7%) of glutamine or lysine in the apical medium was [U-^{13}C; ^{15}N]-glutamine or lysine. For the high apical concentration, concentration in the apical medium for the AA studied was 2.05 mmol/L while for low apical concentration, it was 0.5 mmol/L. Ttreatments were named as follows: Gln_High, Lys_High, Gln_Low, Lys_Low. Within each treatment, isotopic analyses were performed after 15, 30, 45, 60, 90, 120 and 240 minutes of incubation of cells with the labelled AA. At each time point, medium from the apical and basolateral medium was sampled, and cells were harvested. Analysis of samples was performed via EA-IRMS, to measure isotopic composition in C and N of samples. Sampling procedure was destructive for transwells, thus different transwells were used per time point and duplicate.

Cell culture

IPEC-J2 cells were purchased from DSMZ (ACC-701, Braunschweig, Germany). Cells were grown on 75 cm^2 flasks (Thermo Scientific, catalog no. 156800) in Dulbecco's Modified Eagle Medium/Ham's F-12 containing L-Glutamine and Sodium bicarbonate (DMEM/Ham's F-12, Gibco catalog no. 11320-033) supplemented with 10% porcine serum (PS, Gibco, catalog no. 26250-084) filtered, decomplemented, and containing 1% penicillin-streptomycin (Gibco catalog no. 15070- 063). Flasks were placed in a humidified (95%) incubator at 37°C under 5% CO_2 and culture medium was replaced every two to three days. When cells reached confluence in one flask, the medium was removed and the cell layer was rinsed before being incubated for 10 min in 5 mL of Trypsin-EDTA 0.25% (Gibco, catalog no. 25200-072). After incubation, cells were centrifugated at 600 *g*, at 20°C for 5 minutes and the supernatant was discarded. After centrifugation, cells forming a pellet were resuspended in a culture medium, to be potentially seeded in new flasks, with ≈ $1x10^6$ cells per flask.

For this experiment, cells from passage three were used and seeded on transwell polyester membrane inserts (0.4 µm pore size, 1.1 cm^2 surface area, Starstedt, catalogue no. 83.3931.040), in 12-well plates at a density of 1×10^5 cells per cm^2 (Starstedt, catalogue no. 83.3921.005). Cells were grown for 14 days in an incubator at 37 °C under humid conditions (95%), and 5% CO_2. The medium was replaced every two to three days, with 1 mL in the apical chamber and 2 mL in the basolateral one. The transepithelial electrical resistance (TEER) was measured every few days with Epithelial Voltohmmeter (EVOM3, Friedberg, Germany). TEER values, in $\Omega \times cm^2$, were calculated as follows:

$$TEER = (R - R0) \times A$$

where R is the measured resistance (Ω), R0 the blank resistance (transwell without cells), and A the transwell effective surface (1.1 cm^2). After confirming epithelial cell differentiation by a TEER value > 300 Ω x cm^2, the cell layers were used for the experiment.

Stable isotopes experiments

For the experiment, labelled AA used were [U-^{13}C; ^{15}N]-Glutamine (Sigma Aldrich, catalog no. 607983) and [U-^{13}C; ^{15}N]-Lysine Hydrolchloride (Sigma Aldrich, catalog no. 608041). In

addition, L-glutamine (Gibo, catalog no. 25030-024) and L-Lysine Hydrochloride (Thermo Scientific, catalog no. J62099.22) were also used.

At day 14, plates were removed from the incubator and transwells were placed in new plates according to their time point (one plate per time point). Placing transwells in different plates per time point was done in order to make sure that plates were taken out of the incubator only once during the experiment, for the sampling. For each treatment, one additional transwell per treatment did not receive labelled AA and was used as a blank for isotopic analyses. The entirety of the basolateral chamber was then filled with Roswell Park Memorial Institute 1640 (RPMI, Gibco, catalog no. 21870) supplemented with 2.05 mmol/L L-glutamine and containing 1 % penicillin-streptomycin (Gibco, catalog no. 15070-063). On the apical chamber, 800 µL of medium was removed and replaced with 750 µL of either the same medium as in the basolateral chamber (HighLys, LowLys) or RPMI supplemented with 1 % penicillin-streptomycin. Plates were then placed in the incubator at 37°C, with humidified atmosphere and 5 % CO_2, for 3 hours of pre-incubation. Plates were taken out of the incubator and 50 µL of a solution containing either: for Gln_High, 2.05 mmol/L of L-Glutamine of which 7 % was [U-^{13}C; ^{15}N]-Glutamine; for Lys_High, 2.05 mmol/L of L-Lysine Hydrochloride of which 7 % was [U-^{13}C; ^{15}N]-Lysine Hydrolchloride; for Gln_Low, 0.5 mmol/L of L-Glutamine of which 7 % was [U-^{13}C; ^{15}N]-Glutamine; for Lys_Low, 0.5 mmol/L of Lysine Hydrochloride of which 7 % was [U-^{13}C; ^{15}N]-Lysine Hydrolchloride. For the blank transwells, 50 µL of a solution containing the same concentration of the AA without any label was used. Plates were incubated for 15 to 240 min.

At each time point, corresponding plate was taken out of the incubator and placed on ice. A volume of 800 µL of apical and basolateral medium was sampled and placed in 2 mL Eppendorf tubes, which were directly immersed in cold nitrogen. Then, the remaining medium in apical chamber was discarded and the apical chamber was rinsed twice with 1 mL of Hans balance salt solution (HBSS, Thermo Fisher, catalog no. 14025092). A cell scraper (Starlab, catalog no. cc7600-0220) was used to detach cells from the porous membrane of the transwell. To retrieve scrapped cells, transwells were rinsed twice with 500 µL of molecular biology grade water (Corning, catalog no. 46-000-Cl) and liquid was sampled in a tube immersed in cold nitrogen. This operation was repeated for each of the two replicates (transwells) per time point and for

the seven time points, as well as for the blank transwell. Samples collected were then stored at -76°C.

Sample preparation

Frozen samples were freeze-dried for 72 hours. During freeze drying, pierced caps were place on the top of tubes to allow water to leave. After freeze-drying, the samples were weighed to obtain the dry matter content. Samples containing the medium (apical or basolateral) were ground for 30 seconds at 1300 rotations per minutes with two 2 mm inox beads. A quantity of 2 mg of ground power was then placed in thin capsules. For cell samples, the totality of the freeze-dried material per tube was taken and placed in a tin capsule, due to the limited amount of material available (<1 mg).

Sample analysis via EA-IRMS

Elemental content and the isotope composition were measured on culture medium or cells with a CHONS Elemental Analyzer (EA Vario PYRO cube; Elementar® Elementar, Villeurbanne, France) coupled to an isotope ratio mass spectrometer (IRMS; Isoprime precision, Elementar®). The possible offsets of raw IRMS values were corrected using both international IEAE (Vienna) and home-made standards (a sucrose–methionine–glycine mixture, referred to as SMG, both cross-calibrated against caffeine IAEA-600 and Ag2S IAEA-S-1). The linearity of IRMS response was checked throughout to be less than 0.02 ‰ nA-1, and furthermore, any possible size effect between samples was very small.

Calculations

Isotopic composition was expressed in the conventional delta notation:

$$\text{Delta } (\Delta, \text{ in } ‰) = [(R_{sample} - R_{standard})/R_{standard}\ ] \text{ x } 1000$$

where R is the heavy/light isotope ratio, and standard material are international reference materials (V-PDB, air N2 for ^{13}C, ^{15}N respectively). Delta was corrected for the delta of blank samples as followed:

$$\text{Delta } (\Delta, \text{ in } ‰)_{corrected} = \text{delta}_{sample} - \text{delta}_{blank}$$

Where $delta_{sample}$ is the delta measured in the sample and $delta_{blank}$ is the delta measured in corresponding non-enriched sampled (apical medium, basolateral medium or cells). Thus, any values above zero signify a net enrichment.

Data preparation

Delta at time zero was added to the dataset, at a value of 0 ‰, based on the enrichment on blank samples. Measured $\Delta^{13}C$ and $\Delta^{15}N$ in basolateral medium was highly variable, with for a given treatment, values higher in the first time points than at time 240 minutes. This was not considered biologically reasonable, and could be attributed to a lack of integrity of cell layers. Thus, values of $\Delta^{13}C$ and $\Delta^{15}N$ in the time period, for a given treatment, which were above $\Delta^{13}C$ and $\Delta^{15}N$ at time 240 minutes were excluded from the analysis. For the treatment Gln_Low, due to a problem in during the experimental procedure, part of the apical medium contaminated the basolateral chamber for time points 15 to 60 min. Thus, these time points for Gln_Low were also excluded from the analysis.

Results

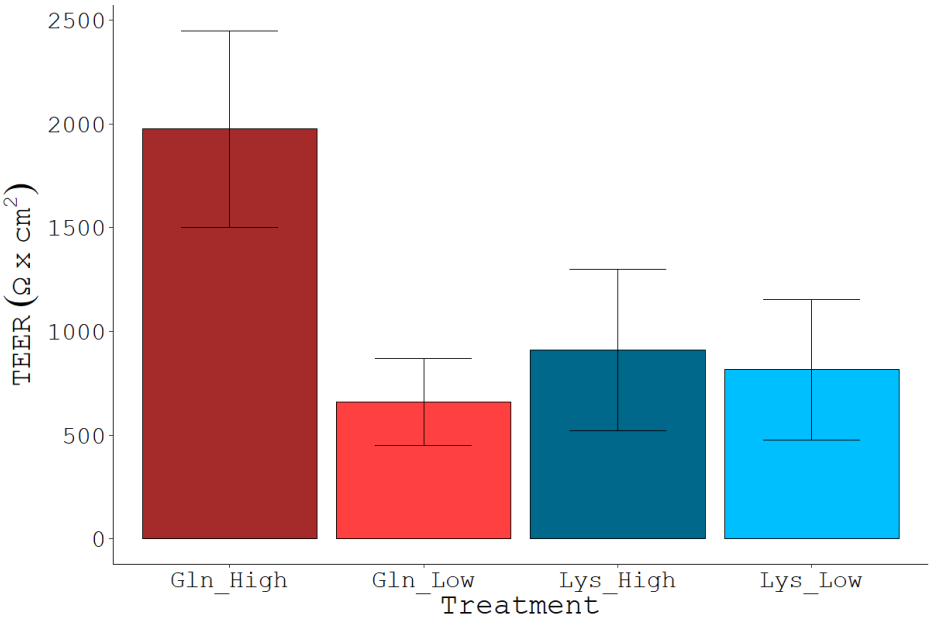

Figure 1. Transepithelial electrical resistance (TEER) in Ω x cm^2, at day 13 of culture of IPEC-J2 cells per treatment. Values are expressed as mean ± standard deviation (n = 20/treatment).

Figure 1 illustrates that the TEER at day 13 was above 300 Ω x cm^2 – which was the lower threshold – for each treatment on average. At day 13, the TEER was higher for the Gln_High treatment (1975 Ω x cm^2) than for the other treatments, for which TEER was around 800 Ω x cm^2. For all treatments, variability in TEER in between transwells was observed, as illustrated by the high standard deviation.

Isotopic composition

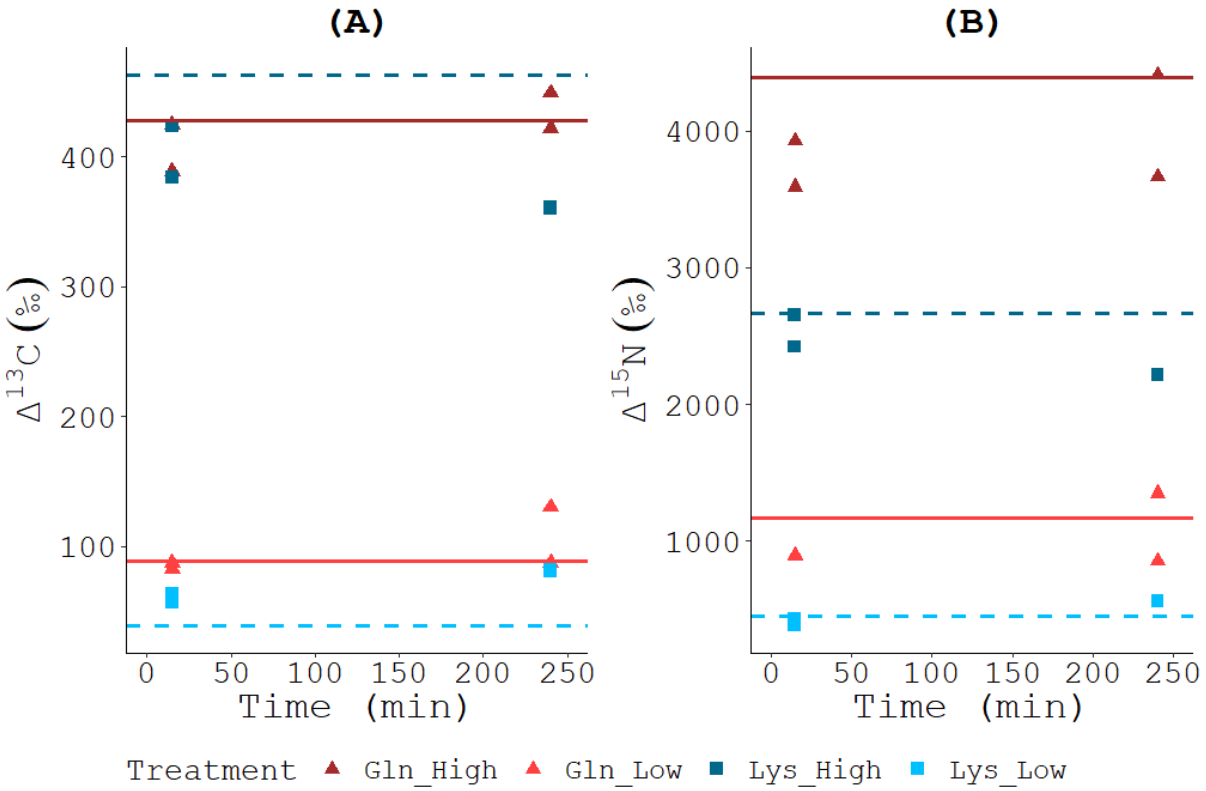

Figure 2. Isotopic composition in $\Delta^{13}C$ (‰, A) or $\Delta^{15}N$ (‰, B) in the apical medium, after 5 and 240 minutes of incubation. IPEC-J2 cells were incubated with in the apical medium, either [U-^{13}C;^{15}N]-glutamine (red triangles) or [U-^{13}C;^{15}N]-lysine (blue squares). With a high (deep colours) or low (light colours) apical concentration in the labelled AA. Lines correspond to the initial enrichment in the apical medium at time zero. Line type corresponds to the labelled AA (plain glutamine, dashed lysine), colour code is the same as for the marks. Values above zero signify a net enrichment.

In the apical medium, both $\Delta^{13}C$ and $\Delta^{15}N$ was similar at time 15 and 240 minutes for each treatment (**Figure 2**). Enrichment was higher for the treatments with a high apical concentration (Gln_High, Lys_High), due to the higher concentration in glutamine or lysine in the apical

medium. For each given treatment, the delta in the apical medium was ≈10 times lower for ^{13}C than ^{15}N. Except for the Lys_High treatment, calculated initial $\Delta^{13}C$ or $\Delta^{15}N$ at time zero (lines) was in close range with measured values (**Figure 2A**).

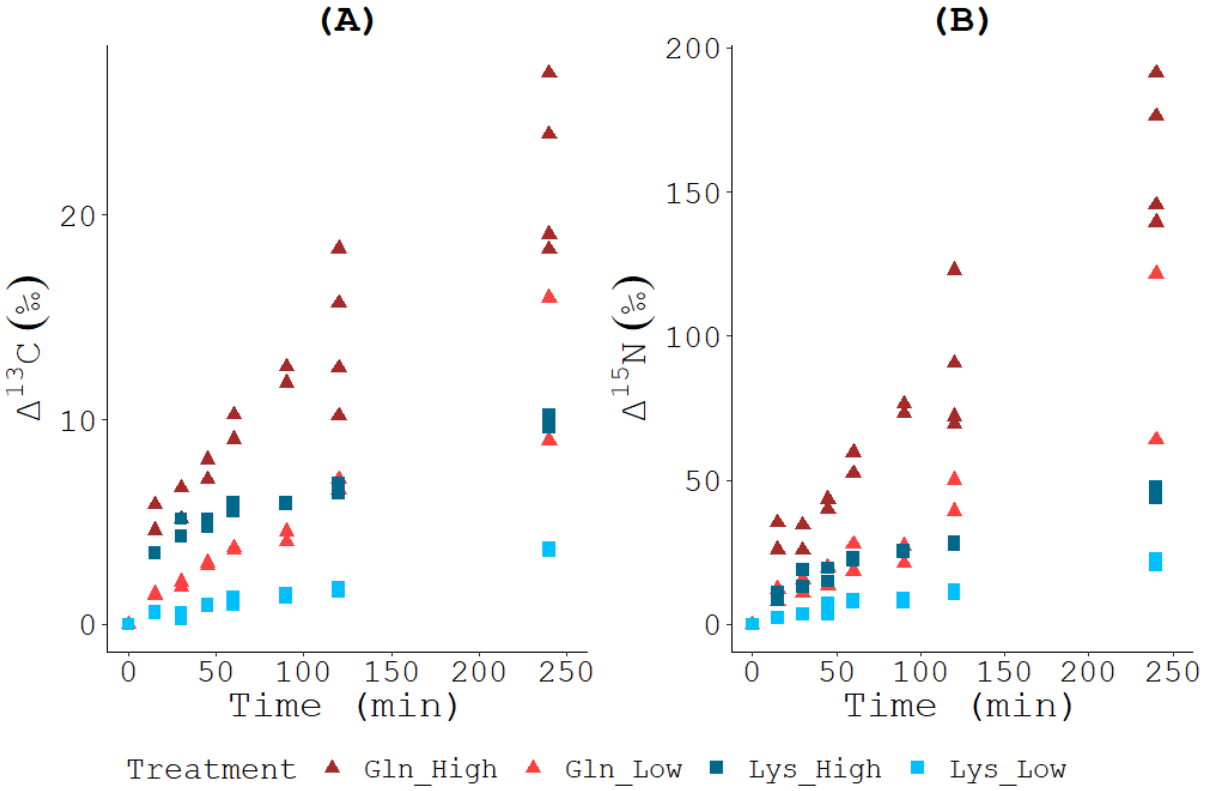

Figure 3. Isotopic composition in $\Delta^{13}C$ (‰, A) or $\Delta^{15}N$ (‰, B) in the cells, during a period of 240 minutes after incubation. IPEC-J2 cells were incubated with in the apical medium, either [U-^{13}C;^{15}N]-glutamine (red triangles) or [U-^{13}C;^{15}N]-lysine (blue squares) with a high (deep colours) or low (light colours) apical concentration in the labelled AA. Values above zero signify a net enrichment.

For each treatment, the $\Delta^{13}C$ and $\Delta^{15}N$ in the cells increased from 15 to 240 minutes, following what seems to be a linear pattern (**Figure 3**). The increase in $\Delta^{13}C$ and $\Delta^{15}N$ between time 0 to 15 minutes seemed sharper than over the rest of the kinetic, although time 0 was here estimated from blank samples. The rate of increase of $\Delta^{13}C$ and $\Delta^{15}N$ over time seemed higher for glutamine (Gln_High, Gln_Low) than for lysine (Lys_High, Lys_Low), regardless of the concentration gradient. For both $\Delta^{13}C$ and $\Delta^{15}N$, the highest and lowest values of delta after 240 minutes were found for Gln_High and Lys_Low, respectively. Values for Gln_Low and

Lys_High were in close range. Overall, $\Delta^{13}C$ and $\Delta^{15}N$ were respectively around 20 times lower in cells than in apical medium.

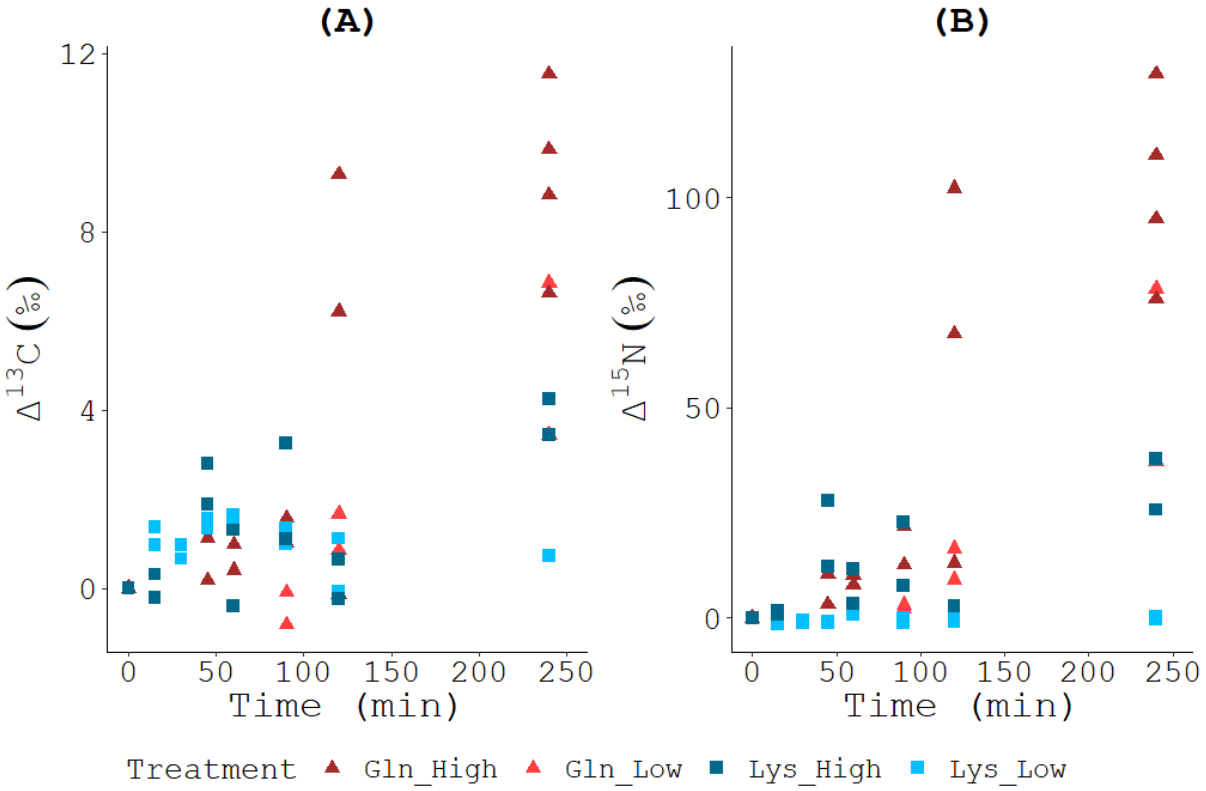

Figure 4. Isotopic composition in $\Delta^{13}C$ (‰, A) or $\Delta^{15}N$ (‰, B) in the basolateral medium during a period of 240 minutes after incubation. IPEC-J2 cells were incubated with in the apical medium, either [U-^{13}C;^{15}N]-glutamine (red triangles) or [U-^{13}C;^{15}N]-lysine (blue squares) with a high (deep colors) or low (light colors) apical concentration in the labelled AA. Values above zero signify a net enrichment.

Care must be taken in interpreting values obtained in the basolateral medium, due to the observed variability as well as the fact that certain points had to be deleted prior to analysis. In the basolateral medium, no clear trend in the evolution of $\Delta^{13}C$ and $\Delta^{15}N$ could be observed over time, due to the high variability of measurements (**Figure 4**). For both Gln_High and Gln_Low treatments, $\Delta^{13}C$ and $\Delta^{15}N$ remained low and stable in the first time points, and increased after 120 minutes. For Lys_High and Lys_Low, $\Delta^{13}C$ and $\Delta^{15}N$ remained stable over time. For the Lys_High treatment, enrichment in ^{13}C and ^{15}N was found in basolateral medium after 45

minutes and lightly increased thereafter. For Low_Lys, delta values remained low over time, especially for ^{15}N, for which almost no enrichment was detected ($\Delta^{15}N < 1‰$).

Delta in percentage of apical delta at time zero

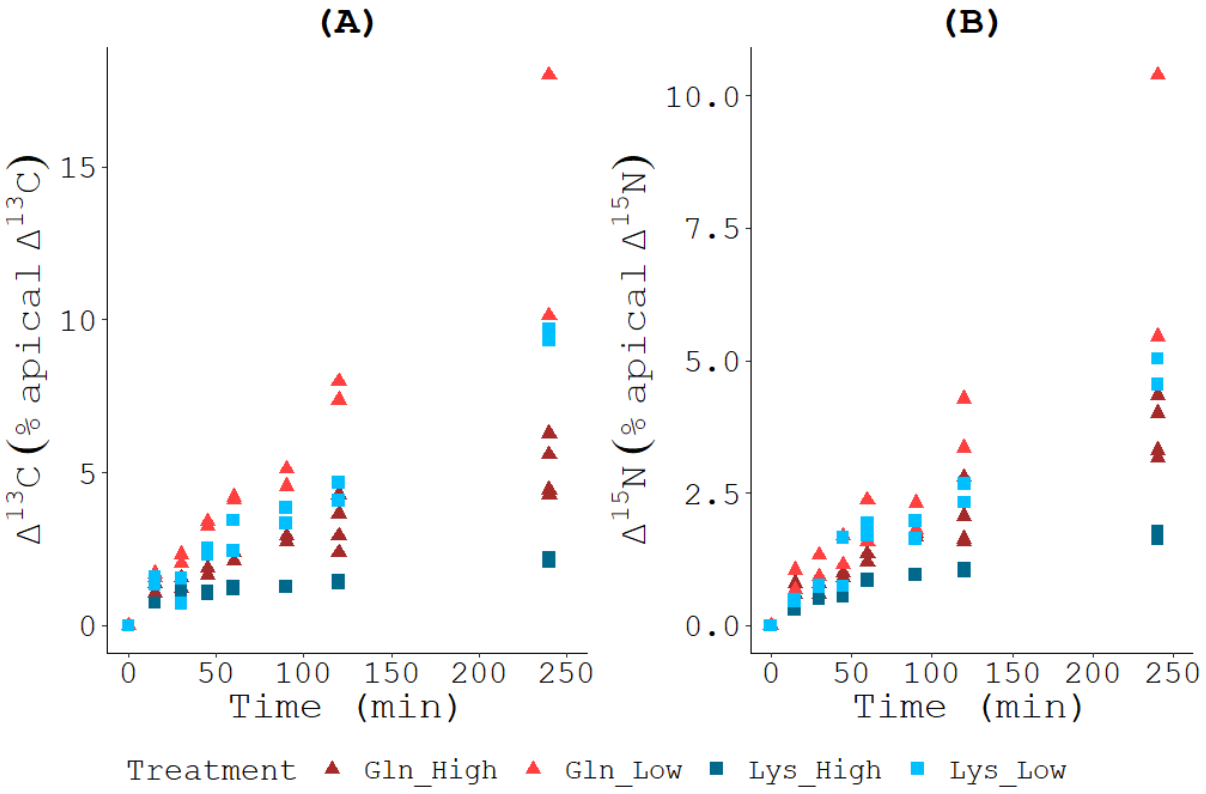

Figure 5. Isotopic composition $\Delta^{13}C$ (A) or $\Delta^{15}N$ (B) in the cells as expressed in percentage of apical $\Delta^{13}C$ or $\Delta^{15}N$ at time zero, during a period of 240 minutes after incubation. IPEC-J2 cells were incubated with in the apical medium, either [U-^{13}C;^{15}N]-glutamine (red triangles) or [U-^{13}C;^{15}N]-lysine (blue squares) with a high (deep colors) or low (light colors) apical concentration in the labelled AA.

Expressing delta in cells in percentage of apical delta at time zero was done in order to observe any potential saturation of apical absorption of AA by the IPEC-J2 cells. Expressing delta in cells in percentage of apical delta at time zero changed the ranking in between treatments (**Figure 5**). In fact, after 240 min, the delta was highest for Gln_Low, closely followed by

Lys_Low, then Gln_High and Lys_High. The ranking in between treatments was the same for both ^{13}C and ^{15}N.

Discussion

To our knowledge, this is one of the first study aiming to investigate metabolic fate of AA incubated with intestinal cells cultivated on transwells over time. In this study, the fate of ^{13}C and ^{15}N from uniformly labelled glutamine and lysine was investigated, at two different apical concentrations. Results obtained showed that cells absorbed both labelled AA present in the apical medium and that part of the ^{13}C and ^{15}N from these AA passed to the basolateral medium. While appearance of ^{13}C and ^{15}N the cell layer seemed to follow a linear pattern over the time period of 240 minutes, passage in basolateral medium was highly variable. Further investigation is required to draw firm conclusions from these results.

Culture of IPEC-J2 cells

In this study, we cultivated the IPEC-J2 cells on transwells for 14 days, in medium containing 10 % PS and at passage three. Our aim was to use similar conditions as colleagues using also IPEC-J2 cells (Perruchot et al., 2025) with the idea that standardised culture conditions allow easier comparisons in between studies (Faria et al., 2024). The use of PS in medium for culturing IPEC-J2 cells was demonstrated to offer closer physiological properties to pigs jejunal cells compared to other serums such as fetal bovine serum (Zakrzewski et al., 2013). During the cell culture period, we monitored the TEER as an indicator of integrity and permeability of the cell layers. However, variability was observed between transwells and especially among treatments, with a much higher TEER at day 13 for Gln_High treatment (1975 Ω x cm^2) than other treatments ($\approx$ 800 Ω x cm^2), while culture conditions were identical. In a previous study performed in our laboratory, TEER values were on average much higher than ours at day 14 (3391 Ω/cm^2) with identical culture conditions (Perruchot et al., 2025). Thereby, biological interpretation of variations in TEER values remains unclear with the IPEC-J2 cell line. We hypothesised that variability observed in TEER measured could explain variability in $\Delta^{13}C$ or $\Delta^{15}N$ observed basolateral medium over time, as TEER is an indicator of the permeability of cell layers. However, we found no correlation in between TEER at day 13 and in $\Delta^{13}C$ or $\Delta^{15}N$ in basolateral medium (p-value>0.05). Further investigation on the elements determining TEER values in transwell IPEC-J2 culture are required to interpret measured values.

Absorption of labelled amino acids from the apical medium by IPEC-J2 cells

In this study, isotopic composition of samples was measured via EA-IRMS and expressed as delta, to follow the fate of ^{13}C and ^{15}N from labelled AA at different time points. Measurement of delta is based on the ratio (*R*) in between a heavy and rare (e.g., ^{13}C) and light and abundant (e.g., ^{12}C) isotope. The deviation of this ratio *R* toward the ratio of an international standard (e.g., Pee Dee Belemnite for C, $^{13}C/^{12}C = 0.011$) is then calculated to obtain the delta. Therefore, values obtained reflect the dilution of the quantity of ^{13}C or ^{15}N in a matrix (apical, basolateral medium or cells), rather than the quantity of the labelled atoms itself. Both $\Delta^{13}C$ and $\Delta^{15}N$ were in a similar range at time 15 and 240 minutes in apical medium, suggesting that delta remained constant in the time period of the experiment. This absence of variation of delta in the apical medium in between the first and last time point suggests that both labelled and unlabeled AA had the same behaviour over this time period. However, enrichment in the apical medium was high for both ^{13}C and ^{15}N ($\Delta^{13}C > 40‰$, $\Delta^{15}N > 350‰$), complicating the interpretation of values obtained. In fact, it is likely that small movement of ^{13}C or ^{15}N would not be detectable in these experimental conditions. There could also have been movement of ^{12}C or ^{14}N present on other molecules during the experiment which would affect measured delta values. To obtain a quantitative estimate of the fraction of ^{13}C or ^{15}N in each matrix per time step, results would have needed to be converted to mass of ^{13}C or ^{15}N. However, it was not possible due to the very limited amount of material collected per transwell, which prevented a precise quantitative estimation. However, orders of magnitudes of measured delta in cells and basolateral medium are 20 to 40 times less than in apical medium. This result suggests that over the time course of the experiment, a small fraction of ^{13}C and ^{15}N from labelled AA passed in the cells or in the basolateral medium.

Appearance of ^{13}C and ^{15}N from glutamine and lysine into IPEC-J2 cells

IPEC-J2 cells progressively absorbed ^{13}C and ^{15}N from glutamine and lysine over time, in a linear manner, as illustrated by the evolution of $\Delta^{13}C$ and $\Delta^{15}N$ during the incubation period (**Figure 3**). Results obtained here showed that over a period of 4 hours, the rate of appearance of ^{13}C and ^{15}N into the cells remained constant which means that cells did not reach a situation of equilibrium during the incubation period. In a situation of equilibrium, fluxes would equilibrate, thus $\Delta^{13}C$ and ^{15}N would stabilise over time. Conceptually, AA incorporated into

the cell layer could be divided into two pools, one of free AA and one of constitutive molecules of the cells (e.g., proteins). The amount of free AA inside the cell is expected to be limited and lower than the quantity of AA into proteins (Garçon et al., 2024); thus accumulation might quickly reach a plateau value. Contrarily, a larger quantity of AA could be incorporated into constitutive molecules such as proteins and that incorporation could take place over a long period of time, as it is known that intestinal cells can renew their protein pool within a day (Sarri et al., 2024). If this property of intestinal cells was maintained with the IPEC-J2 cells cultivated in the set-up used in this experiment, it would require approximately 24 hours to reach a situation of equilibrium in cells. Visually, rate of appearance of ^{13}C and ^{15}N in cells was faster for glutamine than lysine, regardless of the concentration gradient. As glutamine is a major energy source for intestinal cells (He and Wu, 2022), it could be suspected that IPEC-J2 cells have a greater capacity to take up this AA from the apical medium than lysine.

Effect of apical concentration on transport

Values of $\Delta^{13}C$ and $\Delta^{15}N$ in cell layers were higher for the high apical concentration treatments (Gln_High, Lys_High) compared to low apical concentration ones (Gln_Low, Lys_Low). This result indicates that at a high apical concentration of a given AA (2.05 mmol/L) more ^{13}C and ^{15}N appeared into the cell layer than in the low apical concentration situation (0.50 mmol/L). However, results obtained by expressing the delta in percentage of apical delta at time zero showed that in proportion, there was less appearance of ^{13}C and ^{15}N in cells at high apical concentration compared to low apical concentration for a given AA (**Figure 5**). In fact, increasing the apical concentration increased appearance into the cell layer, but to a lower extent than the magnitude of increase in apical $\Delta^{13}C$ or ^{15}N. This result could be attributed to the saturation of active transporters present in the apical membrane of intestinal epithelial cells (Bröer and Fairweather, 2018). At the high apical concentration, concentration of glutamine and lysine was 2.05 mmol/L, which is in range with concentration of glutamine usually found in commercial culture mediums (RPMI 1640, DMEM/F12). While low apical concentration treatment was 0.50 mmol/L, which is range with what is usually found for Lysine-Hydrochloride (0.2 – 0.5 mmol/L). Culture media are formulated to be non-limiting in nutrients, which might result in an excess of nutrients in the media compared to the needs of the cells over a period of 4 hours.

Mediums used for culture of intestinal absorptive cells such as DMEM/F12 and RPMI 1640 are rich in nutrients, especially AA which does not facilitate the study of single AA behavior. In fact, there could be competition in between AA for transporters (Bröer and Fairweather, 2018), as well as competition in between substrates for metabolic pathways, for example glutamine and glutamate. Phenomenon of saturation of apical transport potentially observed in this experimental set-up is something that is expected to occur in the SI during digestion as well. However, the SI is very long organ in which digesta transit along and can be absorbed further if saturation occurs in one segment (Garçon et al., 2024). Contrarily, in a transwell set-up such as in this experiment, there was no flow of nutrients nor surface enlargement. In fact, only a surface of 1.1 cm^2 was displayed by the monolayer of IPEC-J2 cells for absorption on transwells while SI mucosa has a three-dimensional structure which increases the potential surface for absorption. If we consider the presence of plicae and vili, surface enlargement is around times 10 (Helander and Fändriks, 2014), which considerably increases absorptive capacities. These aspects of surface enlargement and flow of nutrients were not represented in the experimental set-up used here.

Kinetics of carbon and nitrogen

Initially, we suspected that the kinetics of ^{13}C and ^{15}N over time would differ for glutamine, due to its use as an energy source by intestinal cells (He and Wu, 2022). On the other hand, behaviour of ^{13}C and ^{15}N was expected to be identical for lysine, due the fact that this AA should be little metabolised by intestinal cells (Chen et al., 2009). Visually, it was not possible to observe differences in the behaviour of ^{13}C and ^{15}N, in the apical medium, cells or basolateral medium. In fact, evolution of $\Delta^{13}C$ and $\Delta^{15}N$ seemed very similar over time in each of the different matrixes. Evolution of the ratio of $\Delta^{13}C/\Delta^{15}N$ was also investigated, but no clear evolution over time could be observed. The absence of differences in the evolution of $\Delta^{13}C$ and $\Delta^{15}N$ over time does not necessarily means that these two atoms were still attached to the same molecule (i.e., glutamine or lysine) and that they followed the same metabolic fate. As an illustration, if glutamine was catabolised in IPEC-J2 cells, then $\Delta^{13}C$ measured could correspond to ^{13}C from CO_2 molecules which are dissolved in the cytosol. Only a difference in kinetic between $\Delta^{13}C$ and $\Delta^{15}N$ would indicate that the two labelled atoms are not attached together on the same molecule anymore.

Pools of amino acids and proteins inside cells

Results showed a linear increased in $\Delta^{13}C$ and $\Delta^{15}N$ in cells for each treatment. When further investigating these data, it seems that if a regression line was drawn, the intercept would be above zero for each treatment (**Figure 3**). This could possibly be due to a contamination of the cells sampled by ^{13}C and ^{15}N present in the apical medium. In fact, it is possible that the step of rinsing before harvesting the cells, although performed twice per transwells, was not sufficient to remove all the labelled AA present in the chamber. It could also be interpreted as a faster increase in $\Delta^{13}C$ and $\Delta^{15}N$ in between time 0 and 15 minutes than for the rest of the experiment. This fast appearance of ^{13}C and ^{15}N in cells in the first minutes of experiment could correspond to the filling of a pool of free AA inside the cells. The amount of free AA inside the cytosol of intestinal cells is likely to be limited and smaller than the one of proteins. Thus, it could be expected that cells quickly absorbed free AA from the apical medium, until this pool of free AA was filled. While incorporation of labelled AA into proteins is a process that might require longer time; thus explaining the linear kinetic of increase in between 15 to 240 minutes. This theory also raises the question of what drives intestinal cells to absorb or not nutrients from the apical medium, whether it is metabolic demand, saturation of transporters or a mechanism homeostasis as we considered in modelling study (Garçon et al., 2024). This interpretation remains speculative and further investigations would be required to comfort this theory.

Conclusion

This study showed that over a kinetics of 4 hours, IPEC-J2 cells cultivated on transwells absorbed a fraction of the ^{13}C and ^{15}N from glutamine and lysine present in the apical medium. Part of the ^{13}C and ^{15}N from glutamine and lysine appeared into the cell layer and appearance occurred at faster rate for glutamine, possibly due to its use as a major energy source for intestinal cells. Incorporation followed a linear kinetic over the 4 hours, suggesting that equilibrium was not reached during the experiment. In the basolateral medium, results showed no clear pattern over time, possibly linked to differences in between cell layers. Further analysis is required before drawing final conclusions from this experiment.

Chapter 6. General discussion

1. Interest of a mathematical model

In this thesis, a mathematical model of AA fluxes in the SI of pigs was built and used in challenging situations, based on simple assumptions and knowledge from the literature. Somehow, the mathematical model we constructed can be seen as a quantitative review of the literature, as it integrates previously acquired knowledge. The process of construction of the model, with the choice of assumptions and the assessment of their impact on the behaviour are seen as the most valuable outcome of this work. We reflected this process in the two chapters of this thesis dedicated to the mathematical model. In chapter 2, emphasis was put on the general principles, assumptions made and the resulting model behaviour. In chapter 3, we presented refinements and their effect on model behaviour before presenting challenging situations. This was a way to take the reader first into the process of reflection about the core elements of the model, rather than simply analysing the outputs. The interest of this model thus lies in its ability to represent the SI as s system, with different properties and emerging behaviour.

Mathematical models can sometimes be repellent for biologists, because of the mathematical formalism behind it. In addition, using the model to perform simulations or for further refinements would require knowledge of a language programming and/or mathematical modelling, which is not accessible, feasible, or desired for all performing research on digestive physiology. We believe that the value of the model is rather in the understanding of how the metabolism of the small intestine can be represented and how it links with biological reality. It has been more of a conceptual exercise, rather than a tool to make predictions *per se.* Presenting the process of model construction and putting more emphasis on relations between elements than on the mathematical formalism could encourage biologists to appropriate modelling approaches. Also, the fact that the model was built with a principle of simplicity in mind helps the reader to interpret outcomes of the model, while larger models can sometimes be seen as black boxes (van Aken, 2024). The added value of this work lies in exploring behaviour of a biological system (i.e., the SI) and the discussions that it brings.

When a study is performed on an animal such as a pig, the whole biological system (i.e., the animal) is under focus. Chosen aspects are studied (e.g., digestibly, intestinal morphology) and the difficulty in interpreting results obtained comes from the fact that numerous processes occur simultaneously, adding noise but also "feedback loops" (i.e., control) to the measurements. The

modelling work carried in this thesis is an effort to view the SI has a system and apprehend its properties. However, it is a simplified model, in which we choose the elements with the aim to quantify AA metabolism in the SI and its interactions with the digestive process. By being a simplification, the model is based on assumptions with which readers may agree or disagree. Yet, it is such disagreements (or agreements) on how elements of the SI function and interacts that is valuable for the scientific community. It forces researchers with different backgrounds to view the SI as a system and reflect on how an element (e.g., transport systems, endogenous secretions) is integrated in the whole.

The construction of a model allows to aggregate knowledge obtained from different perspectives of research. As an illustration, in the model we used knowledge on AA transport by intestinal cells (Bröer and Fairweather, 2018) as well as endogenous secretions (Nyachoti et al., 1997). The interest in building the model lies in the fact that these properties of the SI are often studied separately and, through modelling, they can be integrated. In fact, a phenomenon such as transport is very complex, involving a combination of different processes - and represents a whole area of study in itself (Fan et al., 2001; Gauthier-Coles et al., 2021). Without going into this complexity, we used an important feature (i.e., saturable transport) to incorporate it in the model. Obviously, this implies a simplification of the state of current knowledge as one could argue that diffusion of AA also occurs in the SI mucosa, which is currently beyond the scope of the model. We were rather interested in knowing how a feature of the SI AA metabolism (saturable transport) integrates with other elements of the model. As an illustration, the model offered the possibility to represent the process of saturable transport along the length of the intestinal segments.

2. Potential for model developments

The model as it stands now can be seen as a basis for further developments. We have included some principles such as the homeostasis or the inclusion of a series of intestinal segments with identical structure. As mentioned above, it would require some skills in mathematical modelling and/or language programming, but the Python code is available online on a repository to be used as a basis. Concepts required for modelling are biological thus accessible for researches in biology, it is only their implementation that requires modelling or language programming skills. The exercise of constructing the model has its value in itself, as it enables to adopt a

system thinking approach. It would be valuable if researchers from different backgrounds (e.g., physiology, nutrition, food science) would reflect on how their knowledge can be integrated in a holistic perspective (i.e., a model). Each assumption about which element to add, other elements with which it interacts and associated parametrisation can be discussed thoroughly.

Obviously, process of model construction by its very nature requires simplification, thus we omitted some aspects of the functioning of the SI and digestive process. As an illustration, we did not consider the rate of gastric emptying, which is modulated by the diet and will in turn determine the entrance of digesta in the SI (Goyal et al., 2019). It could be speculated that, as we observed with variation in meal frequency, changing the pattern of gastric emptying would affect the model outputs. Similarly, the effect of inflammation could be an interesting topic and has been studied *in vivo* with a special emphasis on AA use by the intestine (Rémond et al., 2009, 2011). For example, it could be speculated that inflammation impact integrity of intestinal epithelium, thus allowing a form of passive diffusion of AA. Or, if mucin synthesis is increased in situation of inflammation, maximum rate of endogenous secretions could be increased. This highlights that in view of representing the effect of one mechanism (inflammation) several approaches can be taken. Finally, a potential development of the model could be to adapt it for a given AA, with associated metabolic pathways. This could be done, either by keeping the same structure as now. Or by further refining the model using a stochiometric approach, in view of following the fate of C and N from a given AA in the SI.

3. On the relevance of IPEC-J2 cells as a model of enterocytes

We assessed the feasibility of the use of IPEC-J2 cells to study AA metabolism of intestinal cells. To this end, IPEC-J2 cells were cultivated on transwells to allow the formation of a polarized layer, and uniformly labelled AA (glutamine and lysine) with stable isotopes were inserted in the apical medium. A kinetic over 4 hours was performed, to measure via EA-IRMS where the ^{13}C and ^{15}N from the AA was found over time (apical or basolateral media, cells). To our knowledge, only one study used a similar approach (Sakai et al. (2020) using Caco-2 cells and over a period 24 hours.

Our results showed that over 4 hours, cells absorbed labelled AA present in the apical medium, but this represented a small fraction compared to the amount available in the apical medium. This was illustrated by the fact that $\Delta^{13}C$ and $\Delta^{15}N$ remained in ranges 20 to 40 times lower in

cells and basolateral medium than in the apical medium. By using this cell model, the aim was to reproduce the absorption of AA by of the intestinal cells. Transit of digesta in the small intestine is only 2-4 hours long in growing pig (Wilfart et al., 2007), while the length of the small intestine is around 20 meters; thus the time of contact in between intestinal cells and digesta is very short. It suggests that absorption of nutrients by enterocytes must be very fast, in the time range of a few minutes. As discussed in chapter 5, it is possible that IPEC-J2 cells cultivated on transwells quickly filled their pool of free AA thus, limiting the apical absorption. The question remains then what steers the absorption of AA by enterocytes *in vivo*. Several improvements in the cell culture conditions could be considered to assess their effect on the absorptive capacities of IPEC-J2 cells. One option would be to use a less nutrient rich medium, because commercial culture media are formulated to be non-limiting in nutrients for cells over a few days. Having a lower concentration of nutrients in the medium could be a way forward to study the metabolism of IPEC-J2 cells. Other possibilities would be a constant removal of medium from the basolateral chamber, which could mimic blood circulation or adding a step during which medium is removed from the apical chamber, to mimic the alternating meal pattern. If IPEC-J2 cells are considered as potentially able to express the behavior of normal intestinal cells, then the culture conditions to mimic intestinal epithelium *in vivo* should be further investigated. Cultivation of cells on transwells offer the possibility to mimic the polarization of the intestinal epithelium, but many features of the SI are omitted with these devices. As an illustration, it has already been shown that mechanical movement is beneficial for cell culture (Santoni et al., 2025). *In vitro* cell culture offers the possibility to study the effect of these features separately in controlled conditions. One methodological limitation we faced is the low amount of material which was harvested per well (>10 mg) which did not allow for a quantitative analysis of the fate of ^{13}C and ^{15}N. To avoid this, cells could either be cultivated on larger plates (e.g., 6 wells plates) to have more material per transwell, or by pooling transwells together.

Cell behavior results from the environment in which they are immerged as mentioned above, but also from intrinsic properties of the cells (genes of IPEC-J2 cell lines). To our knowledge, few studies have been performed to characterize IPEC-J2 cells and their phenotype (Schierack et al., 2006; Geens and Niewold, 2011; Zakrzewski et al., 2013). In our studies, we observed a high variation of TEER, which was difficult to explain. This variation suggests that cell layers,

even with identical culture conditions and passage, exhibit differences. Visually, microscopic observations suggested that cell layers are not exactly homogenous in the shape and arrangement of cells that compose them. To assess the adequacy of IPEC-J2 cells as models of enterocytes, comparison of their phenotype with *ex vivo* sampled enterocytes could be a way forward. One issue with commercial cell lines such as IPEC-J2 is that their origin is not clearly known, due to the limited information provided by companies selling them. Lack of transparency on how these cells are obtained hampers their use in research, because it could affect repeatability of experiments.

With the recent gain in knowledge about stem cells, organoids could also offer an interesting model to study intestinal epithelial cells (Beaumont et al., 2021). Protocols for the culture of intestinal organoids are still being developed, which might render them less accessible than commercial cell lines such as IPEC-J2. In fact, their use in laboratory requires more technical knowledge than commercial cell lines and come with a certain economic cost. However, their production, once mastered within a laboratory is more transparent that the one of commercial cell lines. They also offer interesting feature, such as the possibility retain phenotypic properties of the organ from which they are sampled (Mussard et al., 2022). Or the fact that as they originate from intestinal stem cells, they are composed of the various cell types present in the intestinal epithelium. Maybe for further investigation on intestinal metabolism, the use of organoids would be a more relevant choice than IPEC-J2 cells.

4. Synergies between cell culture and modelling

The cell culture and mathematical modelling approaches taken in this thesis differ in the perspectives they offer, one being holistic (mathematical modelling) and the other reductionist (cell culture). In the model, a system view was adopted, in which the SI was summarized by its main biological features regarding AA metabolism. While the cell culture approach specifically studied the kinetics of ^{13}C and ^{15}N from two AA (glutamine and lysine) incubated with intestinal cells. Yet, by providing different points of view on the same issue these methods could complement each other. As an illustration, the model development was done with a limited quantitative knowledge available for parametrization. Certain processes which were represented in the model (e.g., transport of AA, exchanges with blood) are very complicated to quantify *in vivo*. Thus, to further refine parameters of the model, other experimental strategies

have to be used. Cell culture could provide the opportunity to estimate parameters which cannot be obtained *in vivo*.

As an illustration, the relative contribution of passive and active transport systems could be investigated, by the use of molecules which block carrier mediated AA transport systems (Fan et al., 2001). Cultured cells could also be used to determine the size of the pool of free AA inside intestinal cells or protein turnover, as discussed in chapter 5. If the model was further developed for a given AA, cell culture with stable isotopes can be used to estimate specific parameters for this AA. To follow the metabolic fate of a labelled AA, IRMS analysis as performed in this thesis could be coupled with gas chromatography (Abadie et al., 2024). Such a method would allow to quantify in which molecules the ^{13}C and ^{15}N are recovered from the labelled AA, instead of simply measuring the isotopic composition in ^{13}C or ^{15}N in organic matter.

5. Alternatives to animal experiments to study digestive physiology

One of the aims of this thesis was to assess the feasibility of studying digestive physiology and metabolism without animal experiments. Animal experiments are often seen as the gold standard for studying physiology, as it allows to have the response of the whole animal to a condition, and measuring global responses. It also has practical limitations, especially studying a specific mechanism separately because it is integrated in a much larger complexity which can add noise (and control) to measurements. As an illustration, studying AA metabolism by the SI is complicated *in vivo* and many authors focused on splanchnic metabolism of AA (Stoll and Burrin, 2006; Bauchart-Thevret et al., 2011) - which includes the SI among other organs. The interest of studying intestinal cells *in vitro* has been demonstrated by the numerous studies performed by the group of Wu (1998), which provided insights on AA related the metabolic pathways present in intestinal cells. One step further could be achieved by trying to reproduce conditions in which intestinal cells are immerged to assess their behavior during digestive processes. It could be speculated that in the future, research in digestive physiology will combine different experimental methods. Noninvasive animal (or human) studies, such as using magnetic resonance imaging (MRI) could be used to provide kinetic of transit of digesta. Systems using cultured cells would be able to offer insights on processes occurring at the level

of the epithelium. While mathematical models could then be used to integrate data obtained in these experiments as inputs for simulations.

A lot of research in nutrition has been ongoing to better estimate protein and AA digestibility in human (Moughan, 2021) especially in a context of transition from animal to more plant proteins in diets in western countries (Salomé et al., 2020). Better knowledge of the interplay in between proteins and their matrix and the SI – such as developed in the model - would be valuable in this perspective. Especially in humans, experiments are limited and a combination of *in vitro* and *in silico* approaches could be valuable. In this thesis, the IPEC-J2 cell line was used, which originates from the pig. However, compared to Caco-2 cells, they are a non-transformed cell line (vs cancer) and originate from the jejunum (vs colon); thereby it could be an interesting model for research in human nutrition as well.

Additionally, there is an important development of *in vitro* models of digestion, which could potentially be coupled with *in vitro* absorptive models to evaluate digestibility and bioavailability of nutrients (Kondrashina et al., 2023) . The experiments performed in this thesis focused on the metabolism of single nutrients (glutamine and lysine) by absorptive cells. Better understanding of how absorptive cells cultivated *in vitro* metabolize single nutrients would be valuable to complement investigations of the effects of food matrixes and digesta properties.

6. On the importance of first-pass metabolism of amino acids

The quantitative importance of first-pass metabolism was the initial question for this thesis. Several studies using catheterised pigs suggested that close to 50% dietary AA was metabolised by the SI, thus not appearing in the blood during digestion (Stoll and Burrin, 2006).

Rather than quantifying first-pass metabolism in itself, this thesis investigated the driver of metabolic use of AA by the SI. In the model, we quantified a first-pass metabolism, which corresponded to the metabolic use of dietary AA by the intestine, and not only a loss of the AA through catabolism and endogenous losses. The concept of first-pass metabolism was initially established in the pharmaceutical studies, to quantify the fraction of a drug which does not reach its target organ (Cohen et al., 1974). This concept might not be adequate for the metabolic use of dietary AA by the SI for several reasons. First, the metabolic use of AA by the SI can simply lead to a delay in their appearance in the blood, for example when AA are used for the synthesis

of intestinal proteins which are renewed on a daily basis (Sarri et al., 2024). Second, one AA can potentially be metabolised several times by the SI before appearing in the blood. Third, metabolism of AA, including its catabolism, does not necessarily mean a loss of AA as AA can be used for the synthesis of other NEAA (e.g., Alanine). Finally, even EAA are expected to be little metabolised by intestinal cells, exception made to their use for protein synthesis (Chen et al., 2009). However, branched-chain amino acids (BCAA) can be deaminated by intestinal cells, appear in the blood as keto-acids and be aminated again in other tissues to form BCAA. Thus, it is rather a temporary change of form due to intestinal metabolism that is observed, than a loss. All these elements suggests that future studies should investigate metabolic use of AA by the SI in a dynamic manner (in time, space, form) rather than just a fixed fraction taken off by intestinal cells during absorption of dietary nutrients.

7. Application in animal science

Nutrition is a major topic of interest in animal science, due to its economic importance and its direct influence on the environmental impact of animal production (de Quelen et al., 2024). In the future, it could be speculated that farm animals might consume more agricultural by-products in their feed (Van Zanten et al., 2019), which are often fibre-rich. In addition, the use of potentially new plant products linked to agro-ecological practices such as roughages for pigs which are also fibre-rich is being investigated for the future animal diets (Franchi et al., 2024). Fibre has an important interplay with intestinal metabolism and influences nutrient digestibility (Montagne et al., 2003). The conceptual model developed in this thesis could help to better understand how fibrous material has an influence on the digestive process in the SI. As an illustration, it can provide insight on interactions in between fibre (non-digestible material) and endogenous secretions both in terms of time (post meal ingestion) and space (segments of SI).

To better assess the adequacy of these raw material as animal feeds, better knowledge about their digestibility will be needed. In this perspective, a combination of *in vitro* and *in silico* methods could help to better understand the digestive process and help interpreting results obtained *in vivo*. This combination of methods has been developed by Schop et al. (2023) by including *in vitro* obtained ingredients physico-chemical properties in a model of digestion. In

the present thesis, we further investigate the possibility of such combination by combining a model of digestive processes and *in vitro* cultured absorptive cells.

In the search of nutritional strategies to preserve intestinal physiology during critical periods such as weaning, AA are pinpointed as molecules with potentially useful health-related effects (Le Floc'h et al., 2018). Supplementation with glutamine has been identified as a potential nutritional strategy to prevent intestinal atrophy in piglets at weaning (Ji et al., 2019). Work performed here could be useful to better understand the interactions between AA and the intestinal tissue. Isolated intestinal cells provide a tool for specifically studying interactions in between the intestinal epithelium and supplemented AA, while the modelling approach can be used to integrate these concepts at the scale of the whole SI.

8. Beyond animal efficiency

This thesis focused on digestive physiology, and was conducted in a research group dedicated to optimizing nutrition of farm animals. Nutrition is identified by most researchers in animal science as the main driver for environmental sustainability of animal farming (Wu et al., 2014) – especially for pig and poultry. Yet, the author would like in the present paragraph to qualify this general view. Sustainability is generally divided in three pillars – economic, social and environmental – here we focus on the later.

Often, sustainability of animal production is approached by estimating the quantity of CO_2 emitted or energy consumed, per quantity of animal product (Garcia-Launay et al., 2014). These costs are calculated by the use the life cycle analysis (LCA) method which attributes costs of a production process per units of end products (i.e., meat, milk). This method represents production process as a straight line, with inputs and outputs within defined boundaries. The main driver for limiting the environmental impact in these studies being the feed efficiency of animals. Yet, to evaluate the sustainability of animal production systems, this method has severe limitations, especially due to the complexity of agricultural systems which rely more on circular biological processes than straight production lines (e.g., nitrogen cycle, Billen et al., 2021). Limits of LCA to evaluate the sustainability of agricultural systems – especially organic ones – have been summarized by van der Werf et al. (2020). Three main limitations have been pinpointed: the need to include new indicators (e.g., for impact on biodiversity or toxicity), a restricted perspective on function of agricultural systems and the inability to represent indirect

effects. As an illustration, the addition of synthetic AA in pigs diets to reduce protein content has often been identified as a strategy to reduce the environmental impact of pig production, through LCA (Garcia-Launay et al., 2014). However, production of synthetic AA, when by fermentative process, is dependent on the availability of large volume of glucose from sugar beet production, a low energy price for heating fermentative reactors as well as for the transport of synthetic AA from the factory to farms. These aspects can be seriously questioned in a sober world, in which energy consumption would be put under constraint. As well as in which production of sugar from sugar beet cannot be fueled with artificial fertilizers nor protected with pesticides, such as neonicotinoids – for which adverse effects on pollinators have been demonstrated (van der Sluijs et al., 2013). Such complex relationships and dependencies in between elements cannot be studied using LCAs, but rather require a systemic dynamics thinking to apprehend their complexity (Sterman, 2002).

An illustration of the importance given to animal efficiency in research in animal farming, is the fast-growing field of research about precision livestock farming (PLF). This is based on the use of sensors and algorithm to monitor, model and control continuously animal responses in farming environment (Tullo et al., 2019). The use of PLF have even been called a "breakthrough toward sustainability" (Pomar and Remus, 2019), which is in the author's opinion, abusive language. In fact, the idea of developing such methods, especially for feeding strategies, goes in the direction of the current dominant paradigm on improving animal efficiency, with all the above-mentioned limitations. Use of these sensors and algorithm has an economic cost and might push more toward specialization of farms, rather than diversification as indicated by agroecological principles (Dumont et al., 2013, 2020). Finally, the environmental impact of devices and sensors used for PLF usually not evaluated by researchers assessing the environmental impact of these strategies. One recent study performed by researchers on informatics evaluated the environmental impact of digital agriculture (which includes PLF) and showed contrasting results (La Rocca et al., 2024). Studies investigating the interest of PLF strategies should extend the scope of their analysis to consider the cost of digital technologies themselves.

There are research groups which studied sustainability of animal farming by taking a different perspective. For example, researchers from Wageningen University investigated the optimal partitioning of land use in between the production of animal feed or human food, with the

assumption that animals only consume feed that is non-human edible (Van Zanten et al., 2018). Another interesting approach was developed by Harchaoui and Chatzimpiros (2018), which looked at energy consumption by farms in France and considering all sources of energy (i.e., biomass or fuel). This study helps to unravel the dependency of current agricultural systems to external energetical inputs. In addition, the same authors studied nitrogen use efficiency at the scale of agricultural systems in France (Chatzimpiros and Harchaoui, 2023). Their study suggests that improving nitrogen use efficiency by making progress in connecting animal and plant production systems is required, rather than just efficiency at the scale of the animal (i.e., feed conversion ratio). Finally, Benoit and Mottet (2023) studied the effect of energy scarcity on animal farming systems in France and showed that considering scarcity of energy implies to reposition and redistribute animal farming within territories.

All the above-mentioned approaches have in common that there are systemic approaches which are built on the representation of selected elements of agricultural systems. Construction of these models allow represent agricultural production as a system, composed of elements which interacts to result in an emerging behavior. In a world in which sobriety in the use of resources and energy is required, these methods should be favored to guide the construction of adequate agricultural models. Animal farming is more than just producing meat or milk, but encompasses its contribution to biodiversity (Fraser et al., 2022), maintaining landscapes or its inclusion in crop production systems and beyond (Martin et al., 2016). Evaluating sustainability of animal production systems goes beyond animal efficiency for production meat or milk but should also consider these aspects.

9. How research in physiology can fit in perspective of sustainability

Research, as any other human activity, needs to reduce its environmental footprint, which goes beyond CO_2 emissions (Steffen et al., 2015). Hence, it is important for researchers to develop scientific methods which can be used in a sober world. When considering activities which have a negative impact on the environment, scientific research would probably be one of the last cited activities. As an illustration, research in the field of agriculture focused on reducing footprint of agricultural systems, but the footprint of the agricultural research itself is overlooked. The quest of knowledge is often considered as a very “noble” activity, which shouldn’t be constrained due to its huge transforming potential. As an illustration, animal

experiments in biomedical research (Petetta and Ciccocioppo, 2021) or field experiments with GMOs (Kuntz, 2012) are topics on which conflicts in between society and scientific community arisen. The question of the environmental impact of research is for now still almost absent in public debate. Although a few example have recently arisen, for example with the debate on construction of a particle accelerator by CERN in between Switzerland and France (Libération, February 2025). Within the field of academic research, the carbon footprint of research related travels has also been pinpointed has a major burden (Ben-Ari et al., 2024).

The question of what research can be performed while complying to the need for sustainability should be asked. In animal farming, research based on outputs at the farm scale such animal growth, milk production or prevalence of diseases could still be obtained in a sober world. But to understand how they are constructed, diving in the biology of animals is required at some point. The question would then be how to perform research in biology and physiology with a limited impact on the environment (i.e., energy consumption, plastics consumption, CO_2 emissions…etc.). There is probably no obvious answer to this question yet, and different pathways must be explored.

Research performed in this thesis had the advantage of intensifying the use of previously published results, by the development of the mathematical model. Conducting experiments and publishing results has an environmental cost, and integrating these results in a mathematical model is a way to fully give value to these results. In addition, mathematical models, via the system thinking they offer, can be used as a tool to guide the design of future experiments. The cell culture approach taken in this thesis focused on a limited number of measurements - mainly isotopic composition via EA-IRMS. For such exploratory studies, it would easily be tempting to measure numerous parameters in order to better apprehend the behavior of the cell system. For example, one would be tempted to study the presence of AA transporters via gene expression (Bröer and Fairweather, 2018). Yet, without prior hypothesis, such additional results would have added noise to our study without providing further information. It was rather decided to make a thorough use of the isotopic composition data obtained to reach conclusions. Then, based on conclusions made on our data and new hypothesis formulated, further experiments with new measurements could be performed. One major drawback of the cell culture experiments such as the ones performed in this thesis is that they consume a large amount of plastics. As an illustration, in a single day of experiments with stable isotopes as

performed in chapter 5, approximately 120 cones for pipettes were used, partly to avoid contamination with labelled AA.

10. Drivers of agricultural research

Overall, research is evaluated by quantitative indicators (i.e., H-index, impact factor) which tend to push toward increasing the number of publications per author. Obviously, the relevance of these methods for evaluating the quality of research can be questioned (Gingras, 2008). One of the consequences of these indicators is the increase in the number of scientific publications, which has outgrown the increase in the number of scientists (Hanson et al., 2024). This increase in the number of scientific publications can be questioned as it probably hampers the possibility of researchers to keep up with their domain, and maybe more dangerous – to seek for knowledge outside of their domain. In addition, it is likely that there is a direct correlation in between the number of publications within a research group and its environmental impact. Thereby, these elements rise the question on how relevance of research conducted by a research group should be evaluated.

On aspect which can be discussed is the emergence of research questions and subsequent innovation in agricultural systems. Traditionally, research for agricultural systems is organized in a linear way, with scientific knowledge produced within public institutions, further developed by public or private actors and disseminated via public or private technical institutes to farmers (Berthet et al., 2018). Although successful in stimulating the production of food, this innovative path has heavy environmental and socio-ecological side effects and fails to consider agricultural systems in their diversity. It is also a very centralized orientation, in which value of innovation developed by farmers (bottom up) is not well considered.

Recently, research has been performed in opening design and coupled innovation to open to way forward to more integration of actors in the construction of innovative agricultural systems (Meynard et al., 2017; Berthet et al., 2018). As an illustration, a fruitful dialog could be established in between agronomist and animal scientists to design innovative animal-crop production systems, based on their complementarities. The research group in which this thesis was conducted is mainly focused on animal nutrition and performs most of its research without discussion with agronomists while plant material is the base element of animal diets. Articles which involve agronomy and animal science are scarce in scientific literature, except for

grazing ruminants and pasture based systems (Martin et al., 2016). More connection in between discipline would be a challenge from the perspective of how scientific research is established. For example, scientific journals are often discipline oriented and a large credit is given to so called fundamental research, which is expected to study elements outside of the influence of the context in which they are.

In addition to dialog in between scientists from different domains, construction of innovation could also involve other actors such as farmers, retailers of transformers. It would most likely influence the research question investigated and the answers obtained. Answers to scientific question depend on alliances in between actors performing research on the field, as well as their promoters, as illustrated with controversy on land-sharing and sparing in agricultural research (Loconto et al., 2020). Another important aspect to favor disruptive innovation is the promotion innovation niches, which escape the dominant sociotechnical regime (Meynard et al., 2017). To innovate, an element integrated (e.g., a farm) in the dominant system must create connection with other actors (i.e., consumers, transformers…etc.). Without the ability to create connections with other actors, no innovation can last, regardless of its technical feasibility.

This thesis was a partnership in between an industrial actor (Adisseo) and a public research institute (INRAE). This partnership allowed to perform very concept-oriented research, with an innovative approach and without direct involvement in the commercial interest of the company. These partnerships in between private companies and public research institute have proven to be efficient in research over the past years in the field of agriculture. However, further connections with other actors, such as farmers in a participatory perspective remain scarce. Obviously, including numerous actors with different perspective within a research project is a challenge (Meynard et al., 2017). However, including different perspectives (i.e., opening design) could be useful for the choice of research question to be investigated and the perspective taken on results obtained.

General conclusion

The SI has an important metabolic activity, which is expected to interact with digestive processes and influence appearance of AA in the blood. In the present study, we investigated intestinal metabolism of AA using a combination of *in silico* (mathematical modelling) and *in vitro* (cell culture) approaches.

The mathematical modelling work of this thesis was divided in two parts, one describing the model and one refining it and performing challenging simulations. It showed that knowledge is available in literature to build a model of AA metabolism in the SI and its interactions with digestive processes, in pig. The model was constructed with the idea that biological principles on which it is based should be accessible for readers. It provides insight on intestinal metabolism of AA and its interactions with digestive processes. The model challenges concepts of digestibility and first-pass metabolism. It shows that first-pass metabolism, which was considered as a disappearance of dietary AA after intestinal absorption, does not reflect adequately the complexity of intestinal metabolism of AA. Finally, data about AA metabolism in the SI are scarce to parametrise the model, and culture of intestinal cells could be a way forward to provide data.

In this context, this thesis investigated the feasibility of the use cells from the IPEC-J2 line cultivated on transwell to study how intestinal cells metabolise AA. Glutamine and lysine uniformly labelled with stable isotopes (^{13}C, ^{15}N) were incubated in the apical medium and samples of media (apical and basolateral) and cells were taken and analysed for their isotopic composition. Results obtained showed that cells absorbed ^{13}C and ^{15}N from glutamine and lysine present in the apical medium, presumably at a faster rate for glutamine. No equilibrium was reached after 4 hours of incubation suggesting that cells did not reach steady state situation. Appearance of ^{13}C and ^{15}N in the basolateral medium showed no clear trend over time, possibly due to impairment in the integrity of cell layers. These experiments were a first step in setting up a method to use in *vitro* cultivated intestinal cells to study their metabolism as a polarised layer, over time.

Altogether, these results suggest promising perspectives to study digestible physiology using approaches which are alternatives to animal experiments. In this thesis, some links were made in between both approaches, but further work could be conducted in this direction.

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Appendix 1. Courses and involvements during the PhD.

Year	Title	Format	Hours equivalent
2022-2023	Scientific integrity in research	Online (MOOC)	15
2022-2023	Python: from fundamentals to advanced concepts	Online (MOOC)	15
2022-2023	« Sit-down and write your thesis" Methodology and motivation for scientific writing	Online	2
2022-2023	Advanced statistics and linear models	In person	21
2022-2023	Papirus for PhD students (bibliography)	Online	35
2022-2023	Food digestion *in vitro* methodologies and future challenges	In person	80
2022-2023	Organiser of the PhD day within the laboratory	In person	5
2022-2023	Organiser Pint of Science Rennes (popular science)	In person	10
2023-2024	Basics of management and communication	In person	14
2023-2024	Speaking in public	In person	7
2022-2024	Member of the council of the doctoral school EGAAL	In person	10

Appendix 2. Substantial summary of the PhD thesis in French.

Résumé substantiel de la thèse dans la langue Française

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1 Introduction

Cette thèse a été réalisée dans le cadre d'un partenariat entre l'Institut National de Recherche pour l'Agriculture, l'Alimentation, et l'Environnement (INRAE) et l'entreprise Adisseo. Elle a été réalisée dans le cadre d'une convention Cifre (n° 2021/1920) avec Association Nationale de la Recherche et de la Technologie (ANRT), entre juin 2022 et mai 2025.

Les aliments consommés par les animaux sont composés de macronutriments, qui sont décomposés au cours de leur parcours dans le tube digestif par le processus de digestion. Les nutriments ainsi obtenus peuvent ensuite être absorbés par l'animal et utilisés par différents organes et tissus. Les nutriments peuvent servir d'éléments constitutifs, de sources d'énergie, de précurseurs pour la synthèse d'autres molécules, etc., par les cellules de l'organisme. Une classe importante de macronutriments est celle des protéines, présentes dans tous les organismes vivants et assurant diverses fonctions, de la signalisation à la structure, en passant par la défense contre les agents pathogènes, l'expression des gènes, etc. Les protéines sont constituées d'un assemblage de centaines à plusieurs milliers de nutriments plus petits, appelés acides aminés (AA). Après absorption, ces AA sont réutilisés par l'organisme pour la construction de nouvelles protéines, la synthèse d'autres molécules, comme sources d'énergie, etc. Il est connu que l'absence prolongée de certains AA dans l'alimentation altère les fonctions biologiques essentielles et peut entraîner la mort. L'apport en protéines et en AA est donc essentiel et a été au centre des préoccupations des nutritionnistes, tant chez l'animal que chez l'humain, depuis les premiers isolements d'AA isolés (Rose, 1957).

Lors de la digestion, la totalité des AA alimentaires absorbés transite et est absorbée par l'intestin grêle (IG), ce qui lui confère un rôle clé dans le métabolisme des AA. Ainsi, l'utilisation métabolique des AA par l'IG peut influencer leur apparition dans le sang et leur disponibilité pour le métabolisme post-absorptif. L'activité métabolique est très élevée dans l'IG, comme l'illustrent le remplacement quotidien des protéines intestinales (Sève et al., 1993) ou la consommation d'oxygène du tube digestif, qui représente 20 % de l'utilisation d'oxygène de l'animal (Yen et al., 1989). Cette activité métabolique est régie par les deux principales fonctions de l'IG : la digestion et la protection. D'un côté, le SI absorbe les nutriments alimentaires libérés lors de la digestion et sécrète des enzymes pour l'hydrolyse finale des macronutriments. De l'autre, il protégé l'organisme du monde extérieur, via le maintien d'une barrière protectrice soutenue par une sécrétion de mucus et la présence de nombreuses cellules

immunitaires. C'est la combinaison de ces deux fonctions contrastées sur une surface de 30 m² chez l'adulte humain (Helander et Fändriks, 2014) qui entraîne une demande importante en nutriments, dont les AA.

Outre son intérêt agronomique, le porc a été largement utilisé comme modèle en nutrition humaine (Roura et al., 2016). Il s'agit donc probablement de l'une des espèces animales pour lesquelles la digestion est la mieux connue et sur laquelle de nombreuses études nutritionnelles *in vivo* ont été réalisées. Plusieurs auteurs ont étudié l'influence du SI sur l'apparition des AA dans le sang en utilisant le porc. Ils ont estimé qu'environ 50 % des AA essentiels alimentaires absorbés n'apparaissaient pas dans le sang, étant ainsi considérés comme métabolisés par le SI (Stoll et Burrin, 2006). Ce résultat est intriguant, car l'IG ne représente que 3 à 4 % de la masse protéique chez le porc (Agyekum et al., 2012) ; il serait donc inattendu qu'il métabolise 50 % des apports alimentaires. La quantification de l'utilisation intestinale des AA est très variable, la glutamine étant par exemple entièrement métabolisée et l'alanine synthétisée *de novo* par le SI. La quantification du métabolisme intestinal des AA est compliquée *in vivo* par la présence de deux voies d'apport en nutriments, la longueur de l'IG et la rapidité des processus métaboliques (Leterme et al., 1996). De plus, les expériences *in vivo* visant à étudier le métabolisme intestinal nécessitent des protocoles invasifs qui doivent être limités dans les années à venir pour des raisons éthiques.

La modélisation a été beaucoup utilisée en zootechnie, principalement pour étudier les processus digestifs (Bastianelli et al., 1996) mais aussi le métabolisme post-absorptif (van Milgen et al., 2008). L'IG se situe à l'interface entre la digestion et le métabolisme post-absorptif et contribue à ces deux processus, par exemple via la sécrétion d'enzymes de bordure en brosse ou le maintien de la barrière épithéliale. L'idée de modéliser le métabolisme de l'IG a été introduite par Bannink et al. (2006) et mise en pratique par Mills et al. (2017), en se concentrant sur le métabolisme du glucose par l'IG chez les vaches laitières. En nutrition humaine, la modélisation de l'absorption des nutriments dans l'IG a également été identifiée comme un sujet de recherche intéressant pour de futures recherches (Le Feunteun et al., 2021). Les difficultés mentionnées précédemment pour mesurer le métabolisme intestinal des AA pourraient être en partie surmontées par une approche de modélisation. Seule une quantité limitée de données permettrait de paramétrer un tel modèle, mais les principaux processus biologiques et leur ordre de grandeur sont connus.

Les approches *in vitro* offrent la possibilité de disposer d'un modèle réduit et contrôlé de la couche cellulaire intestinale pour réaliser diverses expériences. Des cellules intestinales en culture ont été utilisées pour mesurer la préférence des cellules intestinales envers différents substrats énergétiques (He et Wu, 2022) ou la capacité des entérocytes à dégrader les AA essentiels (Chen et al., 2009). L'utilisation de cellules intestinales en culture pour étudier le devenir métabolique des AA est, à notre connaissance, encore très limitée, à l'exception d'une étude récente (Sakai et al., 2020). Plusieurs lignées cellulaires intestinales sont désormais largement utilisées et peuvent être cultivées de manière polarisée, afin de mimer l'organisation de la muqueuse intestinale (Hevia et al., 2023). Ces modèles cellulaires offrent un outil prometteur pour étudier le métabolisme intestinal des AA en environnement contrôlé. De plus, ils pourraient constituer une solution pour obtenir des données ou élucider des mécanismes pour des approches de modélisation plus holistiques.

Cette thèse a donc étudié le métabolisme intestinal des AA en combinant une approche *in silico* et *in vitro*. Le premier objectif de ce travail était de mieux comprendre comment le métabolisme intestinal influence l'apparition des AA dans le sang pendant la digestion et interagit avec les processus digestifs. Le deuxième objectif de cette thèse était d'évaluer la faisabilité de méthodes alternatives à l'expérimentation animale pour étudier la physiologie et le métabolisme digestifs chez l'animal.

2 Résumé de la littérature

Cette partie résume les principaux manques de connaissances identifiés dans la revue de la littérature. Cette thèse a été initiée par l'intuition que le métabolisme intestinal des AA influence fortement leur apparition dans le sang pendant la digestion. Comme indiqué précédemment, des études *in vivo* étudié l'influence du métabolisme intestinal des AA sur leur apparition dans la veine porte, avec le concept sous-jacent de métabolisme de premier passage. Le concept de métabolisme de premier passage est issu d'études pharmacologiques et représente la fraction d'un composé d'une molécule qui est métabolisée avant d'atteindre son organe (ou tissu) cible. Dans le cas du métabolisme de premier passage des AA dans l'intestin, il représente la fraction des AA alimentaires métabolisée par le tissu intestinal après absorption, n'apparaissant donc pas dans le sang. Plusieurs groupes de recherche ont utilisé des porcs cathétérisés pour examiner la fraction des AA digestibles retrouvée dans le sang porte après digestion, parfois en utilisant

des AA marqués. Seul un petit nombre de ces études (< 20) ont été réalisées et leurs résultats, bien que soulignant l'importance du métabolisme intestinal des AA, ne permettent pas de quantifier le métabolisme de premier passage. L'un des inconvénients de ces études est que la mise en place de cathéters ne permet pas de distinguer les AA absorbés par l'IG de tous les nutriments absorbés par les organes des viscères, dans le sang. De plus, la rapidité avec laquelle les processus métaboliques se produisent et les AA sont recyclés complique l'estimation de l'utilisation intestinale. D'autres aspects, tels que la longueur de l'IG ou la présence de nombreux types cellulaires remplissant différentes fonctions, compliquent encore la quantification des flux d'AA *in vivo*. Enfin, ces études *in vivo* constituent des approches boîte noire sur le métabolisme intestinal des AA, ne permettant pas réellement d'étudier les processus intestinaux, qui expliqueraient les flux mesurés. Ainsi, l'influence du métabolisme intestinal sur la présence d'AA dans le sang reste floue, tout comme le facteur qui l'influence. De nouvelles méthodologies devraient être utilisées pour explorer et comprendre le métabolisme intestinal. La revue ci-dessus a souligné l'importance des modèles mathématiques en zootechnie et en nutrition. Pourtant, les modèles sont souvent divisés en deux catégories : les modèles du métabolisme post-absorption et les modèles de digestion. L'IG, à l'interface des deux, a reçu peu d'attention pour le moment. Concernant les modèles cellulaires, de nombreuses méthodes et types cellulaires peuvent désormais être cultivés pour étudier le métabolisme intestinal. Cependant, ils n'ont pas encore été utilisés pour quantifier le métabolisme des AA de manière dynamique, sur une couche polarisée de cellules intestinales.

3 Méthodologie générale de la thèse

La partie précédente a mis en évidence les limites des approches *in vivo* pour estimer l'utilisation métabolique des AA par l'IG, et notamment le métabolisme de premier passage. Un autre point concernant ces expérimentations animales sur des porcs est qu'elles sont désormais limitées pour des raisons éthiques. J'explique ici le choix qui a été fait d'utiliser à la fois des approches *in silico* et *in vitro* pour tenter de quantifier le métabolisme des AA dans l'IG.

Les deux méthodes ici utilisées ont un aspect réductionniste, au senns qu'elles ne retiennent qu'une fraction des propriétés d'un système biologique. Ces propriétés conservées sont censées être les plus importantes au regard de l'objectif de la thèse. La littérature ci-dessus a mis en évidence de nombreuses caractéristiques qui ne peuvent pas toutes être implémentées dans un

modèle informatique ou de culture cellulaire. Ainsi, l'un des défis de cette thèse a été de choisir les paramètres les plus importants à prendre en compte pour imiter le métabolisme intestinal des AA, *in silico* et *in vitro*.

Par ailleurs, le terme « modèle » dans les modèles informatiques et cellulaires signifie qu'ils reproduisent un système biologique, mais de manière simplifiée. Ce principe de simplicité est un élément directeur de cette thèse, car le modèle mécaniste et le système de culture cellulaire pourraient être très complexes si l'on prenait en compte l'ensemble des connaissances listées dans la littérature ci-dessus. Cela aurait été techniquement impossible et aurait conduit à une forme de chaos, où les relations de cause à effet entre les éléments seraient confuses. De plus, l'utilisation de ces méthodes (*in vitro* et *in silico*) dans ce contexte étant relativement nouvelle, comme mentionné dans la littérature, la prudence et la simplicité sont requis pour initier une progression. Ainsi, tant pour le modèle que pour la culture cellulaire, l'idée était plus de poser des bases solides que d'être exhaustif.

Dans l'approche de modélisation, nous avons cherché à construire une modélisation mathématique représentant les flux métaboliques des AA dans l'IG. La construction d'un modèle mathématique du métabolisme intestinal des AA nécessite d'intégrer les connaissances actuelles disponibles, partiellement résumées ci-dessus. Cela permet d'agréger et de mettre en perspective différentes fractions de connaissances étudiées séparément et de voir leurs interactions. À titre d'illustration, les connaissances acquises lors d'études *in vitro* et *in vivo* antérieures, à différentes échelles, pourraient être combinées. La construction de modèles permet également d'explorer le fonctionnement d'un système biologique, en l'occurrence l'IG. Dans les études *in vivo* chez le porc, l'IG était souvent considéré comme une boîte noire. Un modèle nous permet, sur la base des connaissances actuelles, de reconstituer les étapes intermédiaires afin de mieux comprendre et expliquer les résultats obtenus *in vivo*. Enfin, la construction de modèles permet de tester des hypothèses sur le fonctionnement des processus au sein du système biologique. À titre d'illustration, nous avons souligné précédemment que, bien qu'il n'existe aucune mesure directe *in vivo*, on estime que la plupart des AA sont transportés via des transporteurs saturables. L'effet de cette hypothèse sur le fonctionnement du métabolisme intestinal pourrait être étudié dans un modèle.

De nombreux éléments ont été mis en évidence dans la littérature, et les plus pertinents doivent être sélectionnés pour le travail de modélisation. Une autre étape importante consiste à représenter les relations entre les éléments du modèle par les équations mathématiques appropriées. Cela consiste à identifier les forces motrices de chaque processus. Enfin, la paramétrisation du modèle constitue la dernière étape. Certains paramètres sont issus de données de la littérature, mais il est probable que beaucoup de données manquent pour complétement paramétriser le modèle. Cela ne doit pas être considéré comme une limite à la modélisation du métabolisme de l'IG, car les deux premières étapes, le choix des éléments appropriés et l'identification des forces motrices, sont les plus importantes du processus de modélisation.

C'est dans ce contexte de manque de données qu'est née l'idée d'une approche *in vitro*, pour compléter l'approche scientifique de cette thèse. Les modèles cellulaires in *vitro* permettent d'étudier le fonctionnement métabolique d'un sous-ensemble de cellules intestinales, dans des conditions fixes et connues. Le modèle mécaniste, quant à lui, est une simplification d'un système biologique réel qu'est l'IG. Il s'agit d'une approche plus spécifique, dans le sens où il permet d'étudier le comportement d'un sous-ensemble de cellules, tandis que le modèle mathématique se situe à l'échelle de l'IG. Les modèles cellulaires sont désormais largement développés et utilisés depuis longtemps pour étudier l'utilisation des AA par les cellules intestinales. Cependant, leur utilisation potentielle comme couche cellulaire polarisée et dynamique reste peu explorée à ce jour. L'idée initiale de cette thèse était de permettre une synergie entre les approches *in silico* et *in vitro*. En effet, les modèles cellulaires pourraient permettre d'obtenir des données manquantes pour la paramétrisation des modèles et impossibles à obtenir *in vivo*, par exemple à l'échelle des cellules. La culture cellulaire pourrait également être une voie d'avenir pour évaluer les relations de cause à effet entre les éléments du modèle, en vue du choix des équations appropriées.

Le modèle cellulaire IEPC-J2 a été choisis pour cette thèse, comme compromis entre la proximité avec notre objet d'intérêt et la facilité de culture. Les cellules IPEC-J2 présentent l'avantage, en tant que lignée cellulaire, d'être relativement faciles à cultiver et d'offrir une bonne réplicabilité. Comparées aux cellules Caco-2, les cellules IPEC-J2 sont des cellules jéjunales de porc non transformées. Ces cellules croissent et se différencient sur plusieurs jours, ce qui me permet de les cultiver sur *transwell*. Comme décrit précédemment, les *transwell*

permettent d'obtenir des cellules formant une couche polarisée, avec un côté apical et un côté basolatéral. Cette propriété du SI peut ainsi être maintenue dans ce système *in vitro*.

L'objectif général de cette thèse était d'améliorer la quantification de l'influence du métabolisme intestinal des AA sur leur apparition dans le sang pendant la digestion. À cette fin, une combinaison de deux approches complémentaires a été choisie : la construction d'un modèle mécaniste et la culture cellulaire. L'autre objectif de cette thèse étant d'évaluer la pertinence d'une combinaison d'approches alternatives à l'expérimentation animale pour étudier la physiologie et le métabolisme digestifs.

4 Modèle mathématique

4.1 Matériel & méthode

4.1.1 Principe général

Le modèle représente le devenir d'un AA générique dans différents pools représentant la localisation (luminale [L] ou intestinale [I]), l'origine de l'AA (alimentaire [D] ou endogène [E]) et la forme (protéine [P] ou AA libre [F]) de l'AA. Ici, le terme « endogène » désigne les AA provenant du tissu intestinal. La séparation des AA en fonction de leur origine a été effectuée afin de quantifier le métabolisme de premier passage (c'est-à-dire la partie des AA alimentaires absorbée et transférée dans le sang telle quelle). Tout au long du texte, le terme « protéine » est utilisé pour décrire l'AA comme une protéine, par opposition à l'AA libre. La structure tubulaire du SI a été représentée par une série d'unités fonctionnelles appelées « segments ». Chaque segment a la même structure, mais opère dans un environnement luminal différent (c'est-à-dire la quantité de protéines et d'AA luminales). Un segment est composé de sept pools d'AA présents sous forme de protéines alimentaires luminales (LDP), d'AA libres alimentaires luminales (LDF), d'AA libres alimentaires intestinales (IDF), de protéines endogènes intestinales (IEP), de protéines endogènes luminales (LEP), d'AA libres endogènes luminales (LEF) et d'AA libres endogènes intestinaux (IEF). Les pools sont reliés par des flux représentant le transfert d'AA et les principales voies métaboliques du métabolisme des AA. Les principales caractéristiques du modèle sont l'absorption saturable d'AA luminales, l'homéostasie des pools de protéines intestinales et d'AA libres (par exemple, via les échanges sanguins) et les sécrétions endogènes induites par la présence de protéines luminales. Les AA luminaux non utilisés dans ces processus sont transférés au segment suivant. Pour paramétrer

le modèle, des données issues de la littérature ont été utilisées et, lorsqu'elles n'étaient pas disponibles, des valeurs plausibles ont été retenues.

4.1.2 Structure et organisation

In vivo, l'entrée des protéines alimentaires et des AA dans la proximale l'IG est contrôlée par la vidange gastrique. Dans le modèle, les AA pénètrent dans l'IG sous forme de protéines (*LDP*) dans le premier segment à intervalles de temps réguliers et à vitesse constante (*Ext→LDP*). L'hydrolyse des protéines alimentaires en AA libres (*LDP→LDF*) a été fixée à une vitesse fractionnelle constante, de sorte que l'hydrolyse soit proportionnelle à la quantité de protéines présentes. Le passage des *LDP* aux segments suivants influence également l'hydrolyse, car il influence la quantité de protéines disponibles pour l'hydrolyse à chaque pas de temps, dans un segment donné. L'absorption des AA libres luminaux vers le tissu intestinal (*LDF→IDF*) a été supposée se produire via des transporteurs saturables du côté apical des entérocytes (Broër et Gauthier-Coles, 2022). Une équation de Michaelis-Menten a été utilisée pour modéliser l'absorption, avec un flux maximal (V_{ma}) et une constante d'affinité (K_{ma}). Une fois absorbés, les AA alimentaires sont utilisés pour synthétiser les protéines intestinales (*IDF→IEP*) ou sont transférés dans le sang (*IDF→Ext*). L'utilisation d'IDF pour l'IEP entraîne donc un changement d'origine (c.-à-d., de l'alimentation à l'endogène) et reflète le métabolisme de premier passage dans le modèle. Les protéines intestinales sont soumises à une hydrolyse constante en AA libres (*IEP→IEF*) pour représenter le renouvellement du tissu intestinal, mais sont également régulées par l'homéostasie (*IEF→IEP*) en supposant une valeur cible de *IEP* (T_{IP}). Le principe de la valeur cible suppose que l'intestin tente de maintenir une quantité cible de protéines, en ajustant les flux de synthèse protéique pour maintenir l'homéostasie intestinale. La valeur de T_{IP} a été déterminée en considérant que l'IG représente 3 % du poids corporel (50 kg) et contient 16 % de protéines. Cette quantité de protéines intestinales a ensuite été répartie entre les segments. Ici, le terme « hydrolyse des protéines » est utilisé pour les protéines intestinales et luminales, car il représente le même processus biochimique à différents endroits. Les flux des AA intestinaux libres vers les protéines intestinales étaient proportionnels à la différence entre la valeur actuelle et la valeur cible *IEP*. La contribution de *IDF* et de *IEF* à la synthèse de *IEP* était proportionnelle à la quantité d'AA dans les pools *IDF* ou *IEF* par rapport à la somme de ces deux pools. L'homéostasie du pool protéique *IEP* est donc le principal moteur des flux, attirant les AA libres lorsque la valeur réelle de *IEP* est inférieure à la valeur cible. La combinaison de

l'hydrolyse des protéines intestinales et de l'homéostasie assure le renouvellement des protéines intestinales.

Les pools d'AA libres (*IDF* et *IEF*) sont également contrôlés par l'homéostasie. Pour *IDF*, cela conduit au transfert des AA vers le sang lorsque la valeur réelle des AA libres intestinaux est supérieure à la valeur cible (T_{IF}). Pour *IEF*, un flux bidirectionnel a été utilisé afin que les AA libres du SI puissent être transférés vers ou depuis le sang. Il a été supposé que la présence de protéines dans la lumière déclenche l'excrétion de protéines endogènes (*IEP*→*LEP*), modélisée par une équation de Michaelis-Menten avec un flux maximal (V_{ms}) et une constante d'affinité (K_{ms}). Le *LEP* et le *LEF* ont subi une hydrolyse et une absorption de la même manière que le *LDP* et le *LDF*. Les segments sont reliés entre eux par des pools luminaux (c.-à-d. *LDP*, *LEP*, *LDF* et *LEF*). Le passage des protéines luminales (c.-à-d. *LDP* et *LEP*) au segment suivant a été défini en tenant compte du temps de rétention moyen des protéines dans l'IG. À chaque pas de temps, la totalité des AA libres luminaux non absorbés (c.-à-d. *LDF* et *LEF*) passe au segment suivant. Le modèle a été construit comme une série de 200 segments identiques de l'IG.

Le modèle a été construit en Python version 3.10. Le système d'équations différentielles ordinaires a été résolu à l'aide de la fonction solve_ivp du package Scipy. Le modèle a été exécuté pendant 2 880 minutes (2 jours) avec un pas de temps de 0,5 minute et en utilisant la méthode de Runge-Kutta d'ordre quatre pour résoudre le système d'équations. Les 1 440 premières minutes ont servi à atteindre un état stationnaire, tandis que les 1 440 dernières ont servi à l'analyse. Pour cette étude, le nombre de segments intestinaux (n_{seg}) a été fixé à 200, afin de trouver un compromis entre temps de calcul et réalisme. Le script du modèle est disponible en ligne (https://doi.org/10.57745/CNWM9W).

4.2 Principaux résultats et discussion

Un modèle conceptuel représentant les flux métaboliques d'AA dans l'IG chez le porc est ici proposé. À notre connaissance, il s'agit de la première tentative d'intégration systématique des connaissances (ou des idées) sur le métabolisme des AA dans l'IG. Malgré la complexité des flux dans le l'IG, il permet de représenter les principaux processus et d'obtenir des résultats comparables aux résultats expérimentaux *in vivo*.

4.2.1 Segmentation

L'IG est un organe tubulaire, dont la composition cellulaire est relativement constante sur toute sa longueur (Boudry et al., 2004). Dans des modèles disponibles dans la littérature, l'IG a été représenté comme une série de bassins, basés sur des considérations morphologiques, allant de deux compartiments pour Bastianelli et al. (1996) à sept pour Rivera del Rio et al. (2022). La difficulté de modélisation de l'IG réside dans sa structure tubulaire. Celle-ci entraîne un décalage temporel entre l'entrée de l'estomac et la sortie de l'iléon (c'est-à-dire une réponse de type « premier entré, premier sorti »). Représenter l'IG comme un nombre limité de compartiments connectés entraîne un mélange des digesta, de sorte que les nutriments entrant en premier ne sortent pas nécessairement en premier. Ces deux processus se produisent sans aucun doute, mais la structure tubulaire et le fonctionnement du SI ne peuvent être représentés correctement en utilisant un nombre limité de compartiments. Ici, une série de 200 compartiments a été utilisée, ce qui est similaire à l'approche de Rivest et al. (2000) qui ont modélisé l'IG comme 50 compartiments, ou à celle d'Usry et al. (1991) avec une série de compartiments de longueur identique. Le modèle actuel a été testé avec un grand nombre de segments (jusqu'à 1 000), mais les différences de résultats étaient numériquement faibles par rapport à l'utilisation de 200 segments. Par conséquent, le choix de 200 segments a été retenu pour des raisons de calcul, mais peut être modifié si nécessaire. L'évolution de l'environnement luminal complique encore la modélisation car elle affecte l'interaction entre le digesta et la paroi intestinale, une interaction qui peut être représentée par une modélisation compartimentale. C'est pour cette raison que l'IG a été modélisé comme un grand nombre de compartiments identiques connectés (c'est-à-dire des segments), et que les différences ne se produisent qu'en raison de différences dans l'environnement luminal. Le segment est donc l'unité fonctionnelle de l'IG et la combinaison de tous les segments conserve les principales fonctions métaboliques de l'IG. On peut affirmer que le niveau d'expression des transporteurs (Eugenio et al., 2023) et l'épaisseur de la couche de mucus varient le long de l'IG (Varum et al., 2010), mais cela n'a pas été représenté dans le modèle pour des raisons de simplicité. Mills et al. (2017) ont modélisé la digestion et le métabolisme des glucides dans l'IG en trois sections principales (c'est-à-dire le duodénum, le jéjunum et l'iléon), subdivisées en 47 sous-sections pour représenter sa structure tubulaire. Cela a permis d'inclure des aspects spécifiques pour chacun des trois segments principaux. Le modèle actuel est plus simple que celui de Mills et al. (2017). En tant que premier

modèle conceptuel du devenir des AA dans le SI, nous avons estimé qu'il était important de se concentrer sur les principales structures et forces motrices. Cela n'exclut pas de poursuivre le développement pour aborder d'autres questions (par exemple, la présence d'autres nutriments, la spécificité des AA, le péristaltisme, les différences physiologiques entre les segments intestinaux), à condition que leur rôle dans le métabolisme intestinal puisse être quantifié et généralisé.

4.2.2 Digestibilité

La digestibilité iléale apparente (DIA) obtenue, de 81 %, constituait une estimation raisonnable par rapport aux études *in vivo* portant sur des sources de protéines hautement digestibles (van der Wielen et al., 2023). Leterme et al. (1996) ont souligné que le recyclage des sécrétions endogènes complique l'estimation de la digestibilité réelle. Ce recyclage rapide des sécrétions endogènes est pris en compte dans le modèle : près de la moitié des AA libres alimentaires absorbés sont utilisés pour la synthèse protéique intestinale, sous l'effet des sécrétions endogènes. À la fin de la simulation, 15 % des AA totaux restants à la fin du dernier segment étaient des AA libres, quelle que soit leur origine, ce qui concorde avec les résultats expérimentaux de van der Wielen et al. (2023). Soixante-neuf pour cent des sécrétions endogènes ont été réabsorbées, ce qui se situe dans la fourchette des résultats obtenus par Grala et al. (1998). *In vivo*, la digestibilité est déterminée par la différence entre l'apport alimentaire en protéines et en AA et ce qui reste à l'extrémité de l'iléon. La matière restant à l'extrémité de l'iléon contient à la fois des matières alimentaires non digérées et des sécrétions endogènes, provenant de sources hétérogènes (par exemple, mucus, enzymes, cellules desquamées). La DIA ne prend pas en compte les sécrétions endogènes, contrairement à la digestibilité iléale standardisée (DIS) ou digestibilité iléale vraie (DIV) qui prend en compte tout ou partie des pertes endogènes apparaissant dans l'iléon terminal. Le système DIS suppose l'existence de pertes endogènes basales proportionnelles à la matière sèche ingérée. Le système DIV, difficile à mesurer expérimentalement (Stein et al., 2007), suppose l'existence de « pertes endogènes spécifiques » induites par les caractéristiques des ingrédients alimentaires. Les deux systèmes ne prennent en compte que les pertes endogènes qui arrivent à l'extrémité de l'iléon terminal, mais ignorent ce qui se passe entre les deux. L'entre-deux est difficile à mesurer expérimentalement, mais peut être pris en compte dans la modélisation conceptuelle, fournissant ainsi une base de réflexion sur les concepts sous-jacents. Ceci peut être important

pour les régimes riches en fibres ou en protéines, afin de comprendre comment ces nutriments interagissent avec le métabolisme intestinal et comment ils affectent la digestibilité.

4.2.3 Sécrétion de protéines endogènes

Dans le modèle, la sécrétion de protéines endogènes dans la lumière intestinale (*IEP→LEP*) était initiée par et dépendait de la présence de protéines alimentaires dans la lumière intestinale. En effet, la présence d'un bol alimentaire dans la lumière intestinale stimule la sécrétion d'enzymes responsables de l'hydrolyse et entraîne également le renouvellement de la couche de mucus ou des cellules intestinales par abrasion (Boisen et Moughan, 1996). Cela a permis de synchroniser la demande en AA pour la synthèse protéique intestinale et l'apport d'AA alimentaires provenant du repas, limitant ainsi le flux d'AA depuis le sang. Moughan et Leenaars (1992) ont estimé que la plupart des sécrétions d'azote endogène se produisent dans les deux premiers tiers de l'IG, principalement en raison de l'arrivée d'azote endogène provenant de l'estomac, de la bile et des sucs pancréatiques dans le duodénum. Dans le modèle, les sécrétions protéiques endogènes étaient uniquement d'origine intestinale, la majorité d'entre elles étant sécrétées dans la première moitié de l'IG en raison de la présence d'une quantité élevée de protéines alimentaires luminales. Seules les sécrétions endogènes d'origine intestinale ont été prises en compte, omettant environ un tiers des sécrétions provenant d'autres organes (Nyachoti et al., 1997). À la fin de l'iléon terminal, la plupart des pertes endogènes sont d'origine intestinale (Souffrant et al., 1993), ce qui suggère que les sécrétions endogènes d'origine intestinale augmentent proportionnellement sur la longueur de l'IG. Les sécrétions endogènes sont un moteur important du métabolisme de premier passage, comme l'illustre l'analyse de sensibilité du modèle.

4.2.4 Hydrolyse des protéines luminales

Le taux d'hydrolyse a été fixé pour obtenir moins de 5 % de protéines alimentaires non hydrolysées à la fin de l'iléon, ce qui correspond globalement aux données in vivo pour les sources de protéines hautement digestibles (Fan et Sauer, 1997). Par souci de simplification, aucune distinction n'a été faite entre les types de protéines, qu'elles proviennent de sources alimentaires différentes ou endogènes (par exemple, mucines, enzymes ou cellules desquamées), et l'hydrolyse des protéines luminales alimentaires et endogènes a été supposée suivre le même taux. La digestibilité iléale des différents ingrédients alimentaires varie

considérablement. De plus, les mucines, qui représentent 25 % des pertes d'azote à la fin de l'iléon, sont plus résistantes à l'hydrolyse que la plupart des protéines alimentaires (van der Schoor et al., 2002). L'inclusion de ces différences de taux d'hydrolyse est réalisable et ne modifierait pas la structure du modèle elle-même, mais nécessiterait des paramètres de modèle supplémentaires ou spécifiques, ce qui dépassait l'objectif de cette étude.

4.2.5 Absorption des acides aminés libres luminaux

Les acides aminés sont transportés de la lumière vers les entérocytes par transport actif et diffusion passive (Fan et al., 1998). Le peu de données quantitatives généralisables disponibles nous a conduit à supposer que le transport s'effectuait uniquement via un système saturable. Par conséquent, dans un SI de 200 segments, les AA libres d'un segment peuvent être confrontés à un système de transport saturé et être transportés vers le(s) segment(s) suivant(s) où ils peuvent être absorbés. Comme indiqué dans le Tableau 4, une augmentation de la capacité d'absorption (c'est-à-dire via V_{ma}) n'a eu que peu ou pas d'effet sur la digestibilité totale du tractus et ne déplacerait l'absorption que vers les parties les plus proximales du SI. Cela indique que la capacité d'absorption du SI n'était pas un facteur limitant pour la digestion dans le modèle. Il n'existe pas de corrélation claire entre la longueur de l'intestin grêle et la consommation d'aliments moins digestibles chez les mammifères (Langer et Clauss, 2018). Si le modèle était utilisé pour des animaux ayant une capacité d'absorption limitée (par exemple, en raison d'un IG court), il est possible que la digestibilité soit réduite, car l'absorption serait déplacée vers des parties plus distales.

4.2.6 Homéostasie

Dans le modèle, l'homéostasie est le principal régulateur des flux dans le tissu intestinal. L'homéostasie, telle que définie par Cannon (1929), représente le processus d'autorégulation par lequel les systèmes biologiques maintiennent leurs conditions internes quasi constantes, tout en s'adaptant à un environnement changeant. Dans le modèle, cela a été réalisé en utilisant des valeurs cibles que les pools intestinaux d'AA (T_{IF}) et de protéines (T_{IP}) tentent de maintenir. Ce mécanisme est illustré à la figure 5, où la quantité d'AA libres intestinaux dans différents segments (c'est-à-dire différents environnements) varie en fonction de leur environnement fluctuant, mais revient toujours à sa valeur cible. Outre la régulation homéostatique, l'hydrolyse des protéines intestinales a été intégrée, assurant ainsi leur renouvellement continu. Si la

quantité d'AA libres intestinaux peut dépasser la valeur cible, la quantité de protéines ne peut pas dépasser cette valeur cible en raison des processus constants d'hydrolyse et de sécrétions endogènes. L'intestin constitue l'interface entre le monde extérieur et l'hôte, un environnement parfois hostile pouvant endommager les tissus intestinaux (par exemple, en raison de toxines et d'agents pathogènes). La combinaison de l'hydrolyse continue des protéines et de l'homéostasie illustre ce mécanisme de réparation de manière très simple. Dans le modèle de Mills et al. (2017), l'échange de glucose avec le sang était représenté explicitement par un processus de diffusion facilité. Par conséquent, la glycémie dans les cellules intestinales résultait de l'équilibre entre les flux d'absorption, d'échange avec le sang et de catabolisme. L'homéostasie du glucose résulte donc des flux, ce qui diffère de l'approche utilisée ici, où les flux résultent de l'homéostasie, ce qui limite également le nombre de paramètres à définir.

4.2.7 Métabolisme de premier passage

Le métabolisme de premier passage a été mesuré comme la part des AA alimentaires n'atteignant pas directement le sang après absorption, car ils sont métabolisés par le tissu intestinal lors de la digestion (Stoll et al., 1998). Le terme « métabolisme » englobe plusieurs devenirs possibles des AA dans le tissu intestinal, notamment le catabolisme, la synthèse protéique, la synthèse de molécules dérivées des AA, la synthèse de novo des AA… Dans le modèle, il a été calculé comme la part des AA libres alimentaires absorbés utilisés par le tissu intestinal pour la synthèse protéique (c.-à-d. utilisés pour le flux *IDF→IEP*). *In vivo*, les AA identifiés comme « non métabolisés lors du premier passage » pourraient avoir été métabolisés plusieurs fois par le tissu intestinal en raison de leur métabolisme intestinal très rapide (Leterme et al., 1996) par rapport à la période de mesure du métabolisme de premier passage. Les AA alimentaires peuvent être absorbés et utilisés plusieurs fois pour la synthèse et l'excrétion de protéines endogènes dans la partie proximale du SI et pourraient même apparaître dans le sang dans les 10 minutes suivant leur première arrivée dans le SI. Ces AA seraient alors identifiés comme « non métabolisés lors du premier passage », même s'ils ont été métabolisés. Le métabolisme rapide implique que seul un « métabolisme de premier passage apparent » peut être mesuré expérimentalement (Stoll et Burrin, 2006). Dans le modèle, les protéines endogènes étaient rapidement recyclées, environ un tiers des AA libres apparaissant dans le sang provenant des premiers segments de l'IG. Il est important de souligner que le métabolisme de premier passage n'entraîne pas nécessairement une disparition des AA pour le reste de l'organisme, mais

plutôt une apparition retardée dans le sang. Cette modification de la cinétique d'apparition pourrait avoir des conséquences métaboliques, comme décrit pour les AA libres et liés aux protéines par Eugenio et al. (2023). Bien que le métabolisme de premier passage n'implique pas de perte d'AA en soi, il entraîne un coût énergétique important lié à la synthèse (et peut-être à l'hydrolyse) et au transport des protéines. Le modèle ne prend actuellement pas en compte explicitement le catabolisme des AA, mais il pourrait être inclus, comme cela a été fait pour l'hydrolyse des protéines. Alors que certains AA non essentiels, comme la glutamine, sont connus pour être largement catabolisés, le catabolisme des EAA semble limité dans les entérocytes (Wu, 1998 ; Chen et al., 2009). Outre le catabolisme, les EAA métabolisés lors du premier passage sont susceptibles d'être principalement utilisés pour la synthèse protéique intestinale (c'est-à-dire les protéines constitutives et sécrétées).

4.2.8 Approche de modélisation

Les résultats du présent modèle ne doivent pas être considérés comme des valeurs absolues, mais comme le résultat d'un modèle reposant sur des hypothèses plausibles (Tedeschi, 2023). L'objectif était d'intégrer les connaissances actuelles sur les principaux éléments du métabolisme intestinal des AA. Son intérêt réside dans sa capacité à représenter les connexions entre les éléments de l'intestin et les propriétés émergentes en termes de site et de dynamique d'absorption et de disponibilité des AA pour les tissus périphériques. En raison de la structure du SI et de la dynamique rapide des processus digestifs, il est difficile, voire impossible, d'obtenir des résultats expérimentaux permettant de déchiffrer et de quantifier les processus sous-jacents. Le développement d'un modèle conceptuel a permis d'établir des connexions plausibles entre les éléments, avec des résultats comparables à ceux observés dans les études expérimentales.

En conclusion, un premier modèle conceptuel a été construit, représentant les processus du métabolisme intestinal des AA le long et à travers l'IG. La représentation de l'IG sous la forme d'un grand nombre de segments a permis de rendre compte de sa structure tubulaire. Les résultats obtenus par simulation soulignent l'importance de la dynamique des processus métaboliques ainsi que les différences de comportement entre les segments intestinaux. Ils suggèrent également que le SI peut métaboliser une fraction importante des AA alimentaires pour les sécrétions endogènes et le renouvellement des protéines, tandis que la majeure partie

de ces AA se retrouve dans le sang en fin de digestion. Le modèle doit être considéré comme un cadre permettant d'explorer le comportement des flux métaboliques des AA dans l'IG et d'identifier les domaines dans lesquels des connaissances supplémentaires devraient être acquises.

5 Culture cellulaire

5.1 Matériel & méthode

5.1.1 Principe général

L'objectif de ce volet de la thèse était d'examiner le potentiel des cellules IPEC-J2 cultivées sur des puits trans pour étudier le métabolisme des AA par les cellules intestinales au fil du temps. Afin d'évaluer la pertinence du modèle cellulaire, le métabolisme de deux AA métabolisés différemment par le SI a été comparé. La glutamine a été étudiée car elle constitue une source d'énergie importante pour les entérocytes (He et Wu, 2022) et comparée à la lysine, un AA essentiel qui ne devrait pas être catabolisé par les cellules intestinales (Chen et al., 2009). De plus, deux gradients de concentration apicale à basolatérale sur l'AA donné ont été testés afin d'évaluer l'effet du gradient de concentration sur l'utilisation des AA par les cellules. Les deux AA ont été marqués uniformément au ^{13}C et au ^{15}N et les échantillons ont été analysés par EA-IRMS pour suivre la cinétique du ^{13}C et du ^{15}N au fil du temps.

Au cours de cet essai, quatre traitements ont été administrés, selon l'AA étudié (glutamine ou lysine) et le gradient apical-basolatéral de l'AA d'intérêt (élevé ou faible). Les traitements ont été nommés comme suit : GlnHigh, LysHigh, GlnLow, LysLow. Pour chaque traitement, une cinétique a été réalisée à sept points temporels : 15, 30, 45, 60, 90, 120 et 240 minutes, avec deux réplicas par temps.

5.1.2 Culture des cellules IPEC-J2

Les cellules IPEC-J2 ont été achetées auprès de DSMZ (ACC-701, Braunschweig, Allemagne). Les cellules ont été cultivées dans des flacons de 75 cm^2 (Thermo Scientific, catalogue n° 156800) dans du milieu Eagle modifié de Dulbecco/Ham's F-12 contenant de la L-glutamine et du bicarbonate de sodium (DMEM/Ham's F-12, Gibco catalogue n° 11320-033) supplémenté avec 10 % de sérum porcin (PS, Gibco, catalogue n° 26250-084) filtré, décomplémenté et contenant 1 % de pénicilline-streptomycine (Gibco catalogue n° 15070-063). Les flacons ont

été placés dans un incubateur humidifié (95 %) à 37 °C sous 5 % de CO2 et le milieu de culture a été remplacé tous les deux à trois jours. Lorsque les cellules ont atteint la confluence dans un flacon, le milieu a été retiré et la couche cellulaire a été rincée avant d'être incubée pendant 10 minutes dans 5 ml de trypsine-EDTA 0,25 % (Gibco, référence 25200-072). Après incubation, les cellules ont été centrifugées à 600 G à 20 °C pendant 5 minutes et le surnageant a été éliminé. Après centrifugation, les cellules formant un culot ont été remises en suspension dans du milieu de culture pour être éventuellement ensemencées dans de nouveaux flacons, à raison d'environ 1 x 10^6 cellules par flacon.

Pour cette expérience, des cellules du troisième passage ont été utilisées et ensemencées dans des inserts de membrane en polyester de type *transwells* (taille des pores : 0,4 µm, surface de 1,1 cm², Starstedt, n° de catalogue : 83.3931.040), dans des plaques à 12 puits, à une densité de 1 × 10 cellules par cm² (Starstedt, n° de catalogue : 83.3921.005). Les cellules ont été cultivées pendant 14 jours dans un incubateur humidifié (95 %) à 37 °C sous 5 % de CO_2. Le milieu a été remplacé tous les deux ou trois jours, à raison de 1 ml dans la chambre apicale et de 2 ml dans la chambre basolatérale. La résistance électrique transépithéliale (TEER) a été mesurée régulièrement à l'aide d'un voltohmmètre épithélial (EVOM3, Friedberg, Allemagne).

5.1.3 Expérimentations avec isotopes stables

Au 14ème jour de culture, les plaques ont été retirées de l'incubateur et les puits transversaux ont été placés dans de nouvelles plaques selon leur point temporel (une plaque par point temporel). Dans la première plaque, correspondant au temps 15 minutes, un puits transversal supplémentaire a été placé pour servir de blanc (un blanc par traitement). La chambre basolatérale a ensuite été entièrement remplie de milieu Roswell Park Memorial Institute 1640 (RPMI) additionnée de 2,05 mmol/L de L-glutamine et contenant 1 % de pénicilline-streptomycine. Dans la chambre apicale, 800 µL de milieu ont été retirés et remplacés par 750 µL du même milieu que dans la chambre basolatérale (HighLys, LowLys) ou de RPMI supplémenté avec 1 % de pénicilline-streptomycine. Les boîtes ont ensuite été placées à nouveau dans l'incubateur à 37 °C, avec une atmosphère humidifiée et 5 % de CO_2, pendant 3 heures de pré-incubation. Après la pré-incubation, les boîtes ont été retirées de l'incubateur et 50 µL d'une solution contenant soit : pour HighGln, 2,05 mmol/L de L-Glutamine dont 7 % étaient de la [U-^{13}C ; ^{15}N]-Glutamine ; Pour HighLys, 2,05 mmol/L de chlorhydrate de L-lysine

dont 7 % de chlorhydrate de [U-^{13}C ; ^{15}N]-lysine ; pour LowGln, 0,5 mmol/L de L-glutamine dont 7 % de [U-^{13}C ; ^{15}N]-glutamine ; pour LowLys, 0,5 mmol/L de chlorhydrate de lysine dont 7 % de chlorhydrate de [U-^{13}C ; ^{15}N]-lysine. Pour les puits transpuits vierges, 50 µL d'une solution contenant la même concentration d'AA sans marquage ont été utilisés. Les plaques ont ensuite été placées à nouveau dans l'incubateur à 37 °C, sous atmosphère humidifiée et avec 5 % de CO_2.

À chaque point temporel, la plaque correspondante a été retirée de l'incubateur et placée sur de la glace. Un volume de 800 µL de milieu apical et basolatéral a été échantillonné et placé dans des tubes Eppendorf de 2 mL, qui ont été directement immergés dans de l'azote froid. Ensuite, le milieu restant dans la chambre apicale a été jeté et la chambre apicale a été rincée deux fois avec 1 mL de solution saline Hanks Balancer Salts Solution (HBSS). Ensuite, un grattoir à cellules a été utilisé pour détacher les cellules de la membrane poreuse du puits trans. Pour récupérer les cellules grattées, les puits trans ont ensuite été rincés deux fois avec 500 µL d'eau de qualité biologie moléculaire et le liquide a ensuite été échantillonné dans un tube immergé dans de l'azote froid. Cette opération a été répétée pour chacune des deux répliques (puits translucides) par point temporel et pour les sept points temporels, ainsi que pour le puits translucide vierge. Les échantillons prélevés ont ensuite été conservés à -76 °C.

5.1.4 Préparation des échantillons

Les échantillons ont ensuite été lyophilisés pendant 72 heures. Avant la lyophilisation, les échantillons ont été placés dans un bain d'azote froid afin d'éviter tout risque de décongélation pendant la préparation. Pendant la lyophilisation, des bouchons percés ont été placés sur le dessus des tubes pour permettre l'évacuation de l'eau. Après la lyophilisation, chaque échantillon a été pesé sur une balance afin d'obtenir sa teneur en matière sèche. Ensuite, les échantillons contenant du milieu (apical ou basolatéral) ont été broyés pendant 30 secondes à 1 300 rotations par minute avec deux billes en inox de 2 mm. Pour les échantillons de milieu, 2 mg de poudre broyée ont ensuite été placés dans des capsules fines. Pour les échantillons de cellules, la totalité de l'échantillon lyophilisé a été prélevée et placée dans une capsule en étain, en raison de la quantité limitée de matériel disponible (< 1 mg).

5.1.5 Analyse des échantillons par EA-IRMS

La teneur en éléments et la composition isotopique ont été mesurées sur milieu de culture ou cellules à l'aide d'un analyseur élémentaire CHONS (EA Vario PYRO cube ; Elementar® Elementar, Villeurbanne, France) couplé à un spectromètre de masse à rapport isotopique (IRMS ; Isoprime precision, Elementar®). Les éventuels décalages des valeurs IRMS brutes ont été corrigés à l'aide des étalons internationaux IEAE (Vienne) et de standards maison (un mélange saccharose-méthionine-glycine, appelé SMG, tous deux étalonnés contre la caféine IAEA-600 et l'Ag2S IAEA-S-1). Il est à noter que la linéarité de la réponse IRMS a été vérifiée tout au long de l'analyse pour être inférieure à 0,02 ‰ nA-1 et que, de plus, tout effet de taille éventuel entre les échantillons était très faible.

5.2 Principaux résultats et discussion

Dans cette étude, nous avons étudié le devenir du 13C et du 15N provenant de glutamine et de lysine uniformément marquées, à deux gradients de concentration différents, incubés avec des cellules IPEC-J2 cultivées sur des puits trans. Les résultats obtenus montrent que les cellules absorbent les deux AA marqués présents dans le milieu apical et qu'une partie du C et du N de ces AA passe dans le milieu basolatéral. Alors que l'incorporation dans la couche cellulaire suit un schéma linéaire sur une cinétique de 240 minutes, le passage en milieu basolatéral est très variable. À notre connaissance, il s'agit de l'une des premières études portant sur le métabolisme quantitatif des AA par une lignée cellulaire cultivée sur des puits trans.

5.2.1 Complexité de la culture de cellules IPEC-J2

Dans cette étude, nous avons cultivé les cellules IPEC-J2 sur des puits trans pendant 14 jours, dans un milieu contenant 10 % de PS et au troisième passage. Notre objectif était d'utiliser des conditions similaires à celles de nos collègues utilisant également des cellules IPEC-J2 (Perruchot et al., 2025) ; avec l'idée que les conditions de culture standardisées permettent des comparaisons plus faciles entre les études (Faria et al., 2024). L'utilisation de PS dans le milieu de culture des cellules IPEC-J2 s'est avérée offrir des propriétés physiologiques plus proches des cellules jéjunales de porc par rapport à d'autres sérums tels que le sérum fœtal bovin (Zakrzewski et al., 2013). Pendant la période de culture cellulaire, nous avons surveillé le TEER comme indicateur de l'intégrité et de la perméabilité des couches cellulaires. Cependant, une

certaine variabilité a été observée entre les *transwells* et surtout entre les traitements, avec un TEER beaucoup plus élevé au jour 13 pour le traitement Gln_High (1975 Ω x cm2) que pour les autres traitements (≈ 800 Ω x cm2), tandis que les conditions de culture et le passage étaient identiques. Dans une étude précédente, des chercheurs du même laboratoire ont obtenu des valeurs qui étaient en moyenne bien plus élevées que les nôtres, au jour 14 (3391 Ω/cm2) également dans des conditions de culture identiques (Perruchot et al., 2025). Par conséquent, l'interprétation biologique des variations de TEER reste floue avec la lignée cellulaire IPEC-J2. Dans la littérature, de nombreux paramètres physiologiques ont été répertoriés comme influençant la mesure de TEER dans les cellules épithéliales en culture, tels que la présence de couches cellulaires multiples (Lu et al., 1996), la forme cellulaire (Grainger et al., 2006) ou la présence de protéines de jonction serrée (Zakrzewski et al., 2013). Nous avons cherché à savoir si la variabilité observée dans le TEER mesuré pourrait expliquer la variabilité du Δ13C ou 15N observé dans le milieu basolatéral au fil du temps, car le TEER est un indicateur de la perméabilité des couches cellulaires. Cependant, nous n'avons trouvé aucune corrélation entre le TEER au jour 13 et $\Delta^{13}C$ ou ^{15}N en milieu basolatéral (valeur de $p > 0,05$). Des recherches plus approfondies sur les éléments déterminant les valeurs de TEER lors de la culture d'IPEC-J2 sur *transwell* sont nécessaires pour mieux interpréter les valeurs mesurées.

5.2.2 Utilisation d'acides aminés utilisée par les cellules IPEC-J2

Les résultats obtenus lors de l'expérience sur les isotopes stables ont montré que $\Delta^{13}C$ et ^{15}N dans le milieu apical sont restés stables pendant les 4 heures d'incubation. Premièrement, ce résultat suggère qu'il n'y a pas eu de fractionnement négatif pour ^{13}C par rapport à ^{12}C, ni pour ^{15}N par rapport à ^{14}N pour l'absorption des AA. Ceci valide l'utilisation d'isotopes lourds stables pour étudier le métabolisme des AA sur des cellules en culture, car ils ne perturbent pas l'absorption des AA par les cellules. Deuxièmement, il semble que ni la [U-^{13}C; ^{15}N]-glutamine, ni la [U-^{13}C; ^{15}N]-lysine ne disparaissent du milieu apical plus rapidement que les autres sources de C et de N (AA, glucose, etc.). En fait, la glutamine et la lysine étaient les principales sources de ^{13}C et ^{15}N dans le milieu apical, et leur disparition aurait entraîné une diminution de $\Delta^{13}C$ et $\Delta^{15}N$ au fil du temps.

Dans les cellules et le milieu basolatéral, $\Delta^{13}C$ et $\Delta^{15}N$ ont augmenté au fil du temps, mais respectivement 20 à 40 fois moins qu'en milieu apical. Ce résultat indique que sur une période

de 4 heures, les cellules IPEC-J2 incubées en milieu RPMI n'ont absorbé qu'une fraction mineure de la quantité de glutamine et de lysine présente dans le milieu apical. Cette absorption limitée de glutamine et de lysine disponibles du côté apical des cellules IPEC-J2 semble contre-intuitive avec le comportement des cellules épithéliales intestinales, censées absorber rapidement de grandes quantités de nutriments. En fait, le transit dans l'IG chez le porc dure environ 4 heures (Wilfart et al., 2007), tandis que l'IG mesure environ 20 m de long, ce qui implique une courte période de contact entre le digesta et les cellules épithéliales intestinales. On pourrait supposer que le fait que les milieux de culture commerciaux tels que le RPMI soient très riches en nutriments - y compris les AA - altère l'absorption de la glutamine et de la lysine, potentiellement par compétition entre les AA pour les transporteurs (Bröer et Fairweather, 2018). On pourrait se demander si cette faible utilisation des AA présents dans le milieu apical est le résultat des conditions de culture ou de la lignée cellulaire elle-même. Les études qui se sont concentrées sur la quantification du métabolisme des AA par des cellules cultivées sur des puits *transwell* sont rares. En utilisant des cellules Caco-2 cultivées dans un milieu riche en nutriments, Sakai et al. (2020) ont montré qu'après 24 heures de culture, les cellules utilisaient moins de 50 % de la plupart des AA présents dans le milieu apical (≈ 5 % de Gln, ≈ 25 % de Lys), à l'exception de l'asparagine et du glutamate qui ont entièrement disparu. On pourrait supposer que dans la présente étude, les cellules IPEC-J2 utilisaient le glutamate plutôt que la glutamine pour répondre à leurs besoins énergétiques, bien que ces deux substrats aient été identifiés comme des sources d'énergie majeures pour les entérocytes in vitro (He et Wu, 2022).

5.2.3 Comparaison de l'absorption de glutamine et lysine

Les cellules IPEC-J2 ont progressivement incorporé du carbone et de l'azote à partir de la glutamine et de la lysine, de manière linéaire, comme l'illustre l'évolution de $\Delta^{13}C$ et ^{15}N pendant la période d'incubation (Figure 3). Les résultats obtenus montrent que sur une période de 4 heures, le taux d'incorporation dans la cellule est resté constant, ce qui signifie que les cellules n'ont pas atteint un état d'équilibre pendant la période d'incubation. En situation d'équilibre, les flux de transport apical et basolatéral s'équilibreraient, de sorte que $\Delta^{13}C$ et ^{15}N resteraient stables dans le temps. Conceptuellement, l'AA incorporé dans la couche cellulaire pourrait être divisé en deux pools : l'un d'AA libre et l'autre de molécules constitutives des cellules (par exemple, des protéines). La quantité d'AA libre à l'intérieur de la cellule devrait être limitée, de

sorte que l'accumulation pourrait rapidement atteindre un plateau. On pourrait supposer que la forte augmentation de $\Delta^{13}C$ et ^{15}N observée entre 0 et 15 minutes d'incubation pourrait être due au remplissage rapide de ce pool d'AA libres. À l'inverse, l'accumulation dans les molécules constitutives des cellules pourrait atteindre des valeurs plus élevées. Il est connu que les cellules intestinales peuvent renouveler leur pool protéique en une journée (Sarri et al., 2024) ; il faudrait donc environ 24 heures pour atteindre une situation d'équilibre si cette propriété était maintenue dans le dispositif in vitro utilisé. Cette accumulation progressive de C et N à partir de la glutamine et de la lysine comme protéines cellulaires pourrait se refléter dans l'augmentation linéaire de $\Delta^{13}C$ et ^{15}N observée au fil du temps dans cette étude.

5.2.4 Effet du gradient de concentration en milieu apical

Les résultats de $\Delta^{13}C$ et ^{15}N pour les couches cellulaires montrent des valeurs plus élevées pour les traitements à gradient élevé (Gln_High, Lys_High). Ce résultat indique qu'à un gradient de concentration plus élevé entre les milieux apical et basolatéral pour un AA donné – ce qui se traduit par une concentration apicale plus élevée – davantage de C et de N sont incorporés dans la couche cellulaire qu'à un gradient faible. Cependant, les résultats obtenus en exprimant le delta en pourcentage du delta apical au temps zéro montrent qu'une augmentation du gradient de concentration diminue proportionnellement l'incorporation dans la couche cellulaire. Ce résultat pourrait être attribué à la saturation des transporteurs actifs présents dans la membrane apicale des cellules épithéliales intestinales (Bröer et Fairweather, 2018). En fait, l'augmentation du gradient de concentration a augmenté l'incorporation dans la couche cellulaire, mais dans une moindre mesure que l'ampleur de l'augmentation du $\Delta^{13}C$ ou ^{15}N apical. Au gradient de concentration élevé, la concentration de glutamine et de lysine était de 2,05 mmol/L, ce qui est dans la gamme des concentrations de glutamine habituellement trouvées dans les milieux de culture commerciaux (RPMI 1640, DMEM/F12) mais plus élevé que ce que l'on trouve habituellement pour le chlorhydrate de lysine (0,2 - 0,5 mmol/L). De manière cohérente, une étude utilisant des cellules intestinales isolées a également montré que la production de métabolites de la glutamine (CO_2, ammoniac) n'a pas augmenté proportionnellement lorsque la concentration de glutamine est passée de 0,5 à 2 mmol/L dans le milieu (He et Wu, 2022).

5.2.5 Pertinence du dispositif expérimental mis au point

On pourrait se demander si l'absorption limitée présumée d'AA observée lors des expériences est contradictoire avec l'absorption rapide attendue de grandes quantités de nutriments par les cellules intestinales *in vivo*. Il est probable que les entérocytes, comme toute cellule, ne peuvent pas accumuler de trop grandes quantités d'AA dans leur cytoplasme. Ainsi, on pourrait supposer que les cellules IPEC-J2 cultivées sur des puits trans, dans les conditions utilisées dans cette étude, ont rapidement accumulé une grande quantité d'AA dans leur cytosol, comme l'illustre la forte augmentation de $\Delta^{13}C$ et $\Delta^{15}N$ entre 0 et 15 minutes. Ensuite, leur cytosol étant déjà rempli, les cellules cesseraient d'absorber l'AA libre du milieu apical. Dans un précédent travail de modélisation mathématique que nous avons développé, un niveau cible d'AA libre dans le cytosol des cellules a été mis en œuvre, contrôlant l'efflux d'AA libre du côté basolatéral des entérocytes (Garçon et al., 2024). Dans le dispositif expérimental présenté ici, le milieu a été inséré dans les chambres au début des expériences et est resté inchangé. On pourrait supposer qu'un flux continu de milieu dans la chambre basolatérale stimulerait l'efflux basolatéral d'AA par les cellules intestinales. Ainsi, si les cellules avaient la capacité de se débarrasser de l'AA absorbée par le milieu apical, elles pourraient en absorber une fraction plus importante au fil du temps. Le fait que les cellules cultivées sur des puits trans présentent une structure bidimensionnelle au lieu de la structure tridimensionnelle avec plicae et vili observée dans l'IG a probablement également altéré l'absorption. En effet, l'IPEC-J2 n'a présenté qu'une surface de 1,1 cm^2 pour l'absorption, alors qu'une structure tridimensionnelle. Si l'on considère la présence de plicae et de vili, l'agrandissement de la surface est d'environ x 10 (Helander et Fändriks, 2014), ce qui augmente considérablement les capacités d'absorption. Cette étude a montré que sur une cinétique de 4 heures, les cellules IPEC-J2 cultivées sur des puits trans absorbent une petite fraction de la glutamine et de la lysine présentes dans le milieu apical. Une partie du carbone et de l'azote de la glutamine et de la lysine est incorporée dans la couche cellulaire, probablement sous forme de glutamine et de lysine. L'incorporation a suivi une cinétique linéaire sur les 4 heures, suggérant que l'équilibre n'a pas été atteint pendant l'expérience. Dans le milieu basolatéral, les résultats n'ont montré aucune tendance claire au fil du temps. Des connaissances plus approfondies sur le métabolisme de nutriments individuels par des lignées cellulaires cultivées sur des puits trans sont nécessaires pour généraliser leur utilisation dans les études de digestion.

6 Discussion générale

Dans cette thèse, un modèle mathématique des flux d'AA dans l'IG du porc a été construit et utilisé dans des situations complexes, en s'appuyant sur des hypothèses simples et sur les connaissances issues de la littérature. Ce modèle mathématique peut être considéré comme une revue quantitative de la littérature, car il intègre les connaissances acquises précédemment. Contrairement à la plupart des études en biologie qui étudient les éléments séparément, il représente le métabolisme de l'intestin grêle comme un système. L'intérêt d'une approche systémique réside dans la possibilité d'observer comment les éléments interagissent entre eux et induisent un comportement émergent. Lors de la construction du modèle, nous avons choisi les éléments à représenter (pools et flux) et leurs interactions (équations). Ainsi, le modèle peut être considéré comme une formulation mathématique de notre interprétation du métabolisme intestinal des AA.

6.1.1 Une approche de modélisation mathématique

Les modèles mathématiques peuvent parfois rebuter les biologistes en raison du formalisme mathématique qui les sous-tend. De plus, leur utilisation pour réaliser des simulations ou des améliorations ultérieures nécessiterait des connaissances en programmation et/ou en modélisation mathématique, que l'on ne retrouve pas dans toutes les équipes de recherche en physiologie digestive. Nous pensons donc que l'intérêt de ce modèle réside davantage dans la compréhension de la représentation du métabolisme de l'intestin grêle et de ses liens avec la réalité biologique. Il s'agit davantage d'un exercice conceptuel que d'un outil de prédiction en soi. Présenter le processus de construction du modèle et mettre davantage l'accent sur les relations entre les éléments que sur leur formalisme mathématique pourrait encourager les biologistes à adopter des approches de modélisation appropriées. De plus, le fait que ce modèle ait été construit selon un principe de simplicité peut aider le lecteur à interpréter plus facilement ses résultats, tandis que les modèles plus grands peuvent parfois être perçus comme des boîtes noires (Aken, 2024). Dans cette thèse, l'accent a été mis davantage sur l'interaction entre les éléments et la représentation du fonctionnement de l'IG (par exemple, la saturation des sécrétions, les systèmes de transport), plutôt que sur les résultats numériques obtenus. La valeur ajoutée de ce travail réside dans l'exploration du comportement d'un système biologique (c'est-à-dire l'IG) et les discussions qu'il suscite. Les approches systémiques telles que celle adoptée ici sont rares en biologie et certains aspects (transport des AA, sécrétions endogènes, transit…)

ont tendance à être étudiés séparément. Le travail de modélisation réalisé dans cette thèse vise à considérer l'IG comme un système et à appréhender ses propriétés. En étant une simplification, le modèle repose sur des hypothèses avec lesquelles les lecteurs pourraient être en désaccord. Pourtant, ce sont ces désaccords (ou accords) sur le fonctionnement et les interactions des éléments de l'IG qui sont précieux pour la communauté scientifique. Cela oblige les chercheurs de différents horizons à considérer l'IG comme un système et à réfléchir à la manière dont un élément (par exemple, les systèmes de transport, les sécrétions endogènes…) s'intègre dans l'ensemble.

La construction d'un modèle permet d'agréger les connaissances issues de différentes perspectives de recherche. À titre d'illustration, nous avons utilisé dans ce modèle les connaissances sur le transport des AA par les cellules intestinales (Bröer et Fairweather, 2018) ainsi que sur les sécrétions endogènes (Nyachoti et al., 1997). L'intérêt de la construction de ce modèle réside dans le fait que ces propriétés de l'IG sont souvent étudiées séparément et pourraient ici être intégrées ensemble. En effet, un phénomène tel que le transport est très complexe et représente un domaine d'étude à part entière (Gauthier-Coles et al., 2021 ; Fan et al., 2001). Sans entrer dans cette complexité, nous avons cherché à utiliser une caractéristique importante (le transport saturable) pour l'intégrer au modèle. Évidemment, cela implique une simplification des connaissances actuelles, car on pourrait avancer que la diffusion des AA se produit également dans la muqueuse de l'IG, mais cela dépasse le cadre de ce modèle. Nous étions plutôt intéressés par la manière dont une caractéristique du métabolisme des AA dans l'intestin (le transport saturable) s'intègre aux autres éléments du modèle. À titre d'illustration, le modèle offrait la possibilité de représenter le processus de transport saturable le long des segments intestinaux. L'intérêt du mode réside donc dans sa capacité à représenter l'IG comme un système, doté de propriétés différentes et d'un comportement émergent.

6.1.2 Les cellules IPEC-J2 comme modèle du métabolisme des entérocytes

Les travaux de culture cellulaire menés dans le cadre de cette thèse visaient à évaluer la faisabilité de l'utilisation des cellules IPEC-J2 pour étudier le métabolisme des AA des cellules intestinales. À cette fin, les cellules IPEC-J2 ont été cultivées sur des puits trans pour permettre la formation d'une couche polarisée, et des AA uniformément marqués (glutamine et lysine) avec des isotopes stables ont été insérés dans le milieu apical. Une cinétique de 4 heures a été

réalisée, afin de mesurer par EA-IRMS la concentration en 13C et 15N des AA au cours du temps (milieu apical ou basolatéral, cellules). À notre connaissance, la seule étude ayant adopté une approche similaire dans la littérature est celle de Sakai et al. (2020), utilisant des cellules Caco-2 et sur une cinétique de 24 heures. Nos résultats ont montré qu'en 4 heures, les cellules absorbaient l'AA marqué présent dans le milieu apical, mais en faible proportion par rapport à la quantité disponible dans le milieu apical. Ceci a été illustré par le fait que Δ13C et Δ15N sont restés dans des plages 20 à 40 fois plus faibles dans les cellules et le milieu basolatéral que dans le milieu apical. En utilisant ce modèle cellulaire, l'objectif était de reproduire le comportement des cellules intestinales envers l'absorption. Le transit dans le SI ne dure que 2 à 4 heures chez le porc en croissance (Wilfart et al., 2007), alors que sa longueur est d'environ 20 mètres ; le temps de contact entre les cellules intestinales et le digesta est donc très court. Cela suggère que l'absorption des nutriments par les entérocytes doit être très rapide, de l'ordre de quelques minutes. Comme discuté au chapitre 5, il est possible que les cellules IPEC-J2 cultivées sur des puits *transwell* aient rapidement rempli leur pool d'AA libres, limitant ainsi l'absorption apicale. La question reste donc de savoir ce qui dirige l'absorption d'AA par les entérocytes in vivo. Plusieurs améliorations des conditions de culture cellulaire pourraient être envisagées afin d'évaluer leur effet sur les capacités d'absorption des cellules IPEC-J2. Par exemple, un retrait constant du milieu de culture de la chambre basolatérale pourrait imiter la circulation sanguine. Ou encore, l'ajout d'une étape de retrait du milieu de culture de la chambre apicale, afin de reproduire le schéma alimentaire alterné. Si les cellules IPEC-J2 sont considérées comme potentiellement capables d'exprimer le comportement des cellules intestinales normales, il convient d'étudier plus en détail les conditions de culture permettant de reproduire l'épithélium intestinal in vivo. La culture de cellules sur des puits *transwell* offre la possibilité de reproduire la polarisation de l'épithélium intestinal, mais de nombreuses caractéristiques du SI sont omises avec ces dispositifs. À titre d'illustration, il a déjà été démontré que le mouvement mécanique est bénéfique pour la culture cellulaire (Santoni et al., 2025). Les cultures cellulaires in vitro offrent la possibilité d'étudier l'effet de ces caractéristiques une par une sur les cellules IPEC-J2. Le comportement des cellules résulte de l'environnement dans lequel elles sont immergées, comme mentionné précédemment, mais aussi de leurs propriétés intrinsèques (gènes des lignées cellulaires IPEC-J2). À notre connaissance, plusieurs études ont été menées pour mieux caractériser les cellules IPEC-J2 et leur phénotype (Zakrzewski et al., 2013 ; Geens et Niewold, 2011 ; Schierack et al., 2006). Dans notre étude, nous avons observé une forte variation du

TEER, sans pouvoir l'expliquer. Cette variation pourrait nous laisser penser que les couches cellulaires, même à conditions de culture et de passage identiques, présentent des différences. Visuellement, des observations microscopiques suggèrent que les couches cellulaires ne sont pas parfaitement homogènes quant à la forme et à l'agencement des cellules qui les composent. Pour évaluer l'adéquation des cellules IPEC-J2 comme modèles d'entérocytes, la comparaison de leur phénotype avec celui d'entérocytes prélevés ex vivo pourrait être une piste à explorer. L'un des problèmes des lignées cellulaires commerciales telles que l'IPEC-J2 est que leur origine n'est pas clairement connue, en raison du peu d'informations fournies par les entreprises qui les commercialisent. Le manque de transparence sur la manière dont ces cellules sont obtenues entrave leur utilisation en recherche, car il pourrait affecter la répétabilité des expériences.

Avec les progrès récents des connaissances sur les cellules souches, les organoïdes offrent également un modèle intéressant pour l'étude des cellules épithéliales intestinales (Beaumont et al., 2021). Les protocoles de culture des organoïdes intestinaux sont encore en cours de développement, ce qui pourrait les rendre moins accessibles que les lignées cellulaires commerciales telles que l'IPEC-J2. En effet, leur utilisation en laboratoire requiert davantage de connaissances techniques que les lignées cellulaires commerciales et entraîne un certain coût économique. Cependant, leur production, une fois maîtrisée en laboratoire, est plus transparente que celle des lignées cellulaires commerciales. Ils présentent également des caractéristiques intéressantes, comme la possibilité de conserver les propriétés phénotypiques de l'organe dont ils sont issus (Mussard et al., 2022). Ou encore, comme ils proviennent de cellules souches intestinales, ils sont composés des différents types cellulaires présents dans l'épithélium intestinal. Pour approfondir les recherches sur le métabolisme intestinal, l'utilisation d'organoïdes serait peut-être plus pertinente que celle des cellules IPEC-J2.

6.1.3 Des méthodes alternatives à l'expérimentation animale pour étudier la physiologie

L'un des objectifs de cette thèse était d'évaluer la faisabilité de l'étude de la physiologie et du métabolisme digestifs sans expérimentation animale. L'expérimentation animale est souvent considérée comme la référence en physiologie, mais elle présente également des limites pratiques. À titre d'exemple, l'étude du métabolisme des AA par l'IG chez l'animal est compliquée et de nombreux auteurs se sont concentrés sur le métabolisme splanchnique des

AA (Bauchart-Thevret et al., 2011 ; Stoll et Burrin, 2006), qui inclut le l'IG parmi d'autres organes. L'intérêt de l'étude des cellules intestinales in vitro a été démontré par les nombreuses études réalisées par le groupe de Wu (1998), qui ont apporté des éclairages sur les voies métaboliques liées aux AA présentes dans les cellules intestinales. Une étape supplémentaire pourrait être franchie en essayant de reproduire les conditions d'immersion des cellules intestinales afin d'évaluer leur comportement au cours des processus digestifs. On peut supposer qu'à l'avenir, la recherche en physiologie digestive combinera différentes méthodes expérimentales. Des études non invasives sur l'animal (ou l'humain), telles que l'imagerie par résonance magnétique (IRM), pourraient permettre de suivre la cinétique du transit des digesta. Des systèmes utilisant des cellules en culture permettraient de mieux comprendre les processus se produisant au niveau de l'épithélium. Des modèles mathématiques pourraient ensuite être utilisés pour intégrer les données obtenues lors de ces expériences comme données d'entrée pour les simulations.

De nombreuses recherches en nutrition sont actuellement en cours afin de mieux estimer la digestibilité des protéines et des acides aminés chez l'homme (Moughan, 2021), notamment dans un contexte de transition vers des régimes alimentaires plus riches en protéines végétales dans les pays occidentaux (Salomé et al., 2020). Une meilleure connaissance de l'interaction entre les protéines, leur matrice et l'IG, telle que développée dans le modèle, serait précieuse dans cette perspective. Chez l'homme en particulier, les expériences sont limitées et une combinaison d'approches *in vitro* et *in silico* pourrait s'avérer précieuse. Dans cette thèse, la lignée cellulaire IPEC-J2, issue du porc, a été utilisée. Cependant, comparée aux cellules Caco-2, il s'agit d'une lignée cellulaire non transformée (contrairement aux cellules cancéreuses) et issue du jéjunum (contrairement au côlon) ; elle pourrait donc constituer un modèle intéressant pour la recherche en nutrition humaine.

De plus, les modèles in vitro de digestion connaissent un développement important, qui pourraient potentiellement être couplés à des modèles d'absorption in vitro pour évaluer la digestibilité et la biodisponibilité des nutriments (Kondrashina et al., 2023). Les expériences réalisées dans le cadre de cette thèse, axées sur le métabolisme de nutriments individuels par des cellules absorbantes, apportent des éclairages utiles dans cette perspective. En effet, avant d'étudier l'effet des matrices alimentaires sur les propriétés du digesta et l'absorption ultérieure

par les cellules intestinales, il est important de comprendre comment ces cellules intestinales cultivées métabolisent les nutriments.

6.1.4 Sur la place de l'efficience alimentaire dans la recherche agronomique

Cette thèse portait sur la physiologie digestive et a été menée au sein d'un groupe de recherche dédié à l'optimisation de la nutrition des animaux d'élevage. La nutrition est identifiée par la plupart des chercheurs en zootechnie comme le principal moteur de la durabilité environnementale de l'élevage (Wu et al., 2014), notamment pour les porcs et les volailles. Cependant, l'auteur souhaite, dans ce paragraphe, nuancer cette vision générale.

La durabilité de la production animale est souvent mesurée par la quantité de CO_2 émise ou d'énergie consommée par quantité de produit animal (Garcia-Launay et al., 2014). Ces coûts sont calculés à l'aide de la méthode d'analyse du cycle de vie (ACV), qui attribue les coûts d'un processus de production par unité de produit fini (viande, lait, etc.). Cette méthode représente le processus de production comme une ligne droite, les intrants et les extrants étant définis. Le principal facteur de limitation de l'impact environnemental dans ces études est l'efficacité alimentaire des animaux. Cependant, pour évaluer la durabilité des systèmes agricoles, cette méthode présente de graves limites, notamment en raison de la complexité des systèmes agricoles qui reposent davantage sur des processus biologiques circulaires que sur des lignes de production droites (par exemple, le cycle de l'azote, Billen et al., 2021). Les limites de l'ACV pour évaluer la durabilité des systèmes agricoles – en particulier biologiques – ont été résumées par van der Werf et al. (2020). Trois limites principales ont été identifiées : la nécessité d'inclure de nouveaux indicateurs (par exemple, pour l'impact sur la biodiversité ou la toxicité), une perspective restreinte sur la fonction des systèmes agricoles et l'incapacité à représenter les effets indirects. À titre d'illustration, l'ajout d'AA synthétiques dans l'alimentation des porcs pour réduire la teneur en protéines a souvent été identifié comme une stratégie pour réduire l'impact environnemental de la production porcine, grâce à l'ACV (Garcia-Launay et al., 2014). Cependant, la production d'AA synthétiques, par fermentation, dépend de la disponibilité d'un volume important de glucose issu de la production de betterave sucrière, d'un faible coût de l'énergie pour le chauffage des réacteurs de fermentation et pour le transport des AA synthétiques de l'usine aux exploitations agricoles. Ces aspects peuvent être sérieusement remis en question dans un monde plus sobre, où la consommation énergétique serait limitée. De plus,

la production de sucre à partir de betterave sucrière ne peut être alimentée par des engrais artificiels ni protégée par des pesticides, tels que les néonicotinoïdes, dont les effets néfastes sur les pollinisateurs ont été démontrés (van der Sluijs et al., 2013). Ces relations et dépendances complexes entre les éléments ne peuvent être étudiées par des ACV, mais nécessitent plutôt une approche dynamique systémique pour appréhender leur complexité (Sterman, 2002).

L'importance accordée à l'efficacité animale dans la recherche en élevage est illustrée par le domaine de recherche en plein essor de l'élevage de précision (PLF). Ceci est basé sur l'utilisation de capteurs et d'algorithmes pour surveiller, modéliser et contrôler en continu les réponses animales en environnement agricole (Tullo et al., 2019). L'utilisation de PLF a même été qualifiée de « percée vers la durabilité » (Pomar et Remus, 2019), ce qui est, de l'avis de l'auteur, un langage abusif. En fait, l'idée de développer de telles méthodes, en particulier pour les stratégies d'alimentation, va dans le sens du paradigme dominant actuel sur l'amélioration de l'efficacité animale, avec toutes les limites mentionnées ci-dessus. L'utilisation de ces capteurs et algorithmes a un coût économique et pourrait pousser davantage à la spécialisation des exploitations, plutôt qu'à la diversification comme l'indiquent les principes agroécologiques (Dumont et al., 2013, 2020). Enfin, l'impact environnemental des dispositifs et capteurs utilisés pour les PLF n'est généralement pas évalué par les chercheurs évaluant l'impact environnemental de ces stratégies. Une étude récente réalisée par des chercheurs en informatique a évalué l'impact environnemental de l'agriculture numérique (qui inclut les PLF) et a montré des résultats contrastés (La Rocca et al., 2024). Les études examinant l'intérêt des stratégies PLF devraient étendre la portée de leur analyse pour prendre en compte le coût des technologies numériques elles-mêmes.

Certains groupes de recherche ont étudié la durabilité de l'élevage sous un angle différent. Par exemple, des chercheurs de l'Université de Wagenignen ont étudié la répartition optimale de l'utilisation des terres entre la production d'aliments pour animaux et la production d'aliments pour humains, en partant du principe que les animaux ne consomment que des aliments non comestibles pour l'homme (Van Zanten et al., 2018). Une autre approche intéressante a été développée par Harchaoui et Chatzimpiros (2018), qui ont examiné la consommation énergétique des exploitations agricoles en France, toutes sources confondues (biomasse ou combustibles). Cette étude permet de comprendre la dépendance des systèmes agricoles actuels aux apports énergétiques externes. De plus, les mêmes auteurs ont étudié l'efficacité de

l'utilisation de l'azote à l'échelle des systèmes agricoles en France (Chatzimpiros et Harchaoui, 2023). Leur étude suggère qu'il est nécessaire d'améliorer l'efficacité de l'utilisation de l'azote en progressant dans la connexion des systèmes de production animale et végétale, plutôt qu'en se concentrant uniquement sur l'efficacité à l'échelle de l'animal (c'est-à-dire l'indice de conversion alimentaire). Enfin, Benoit et Mottet (2023) ont étudié l'effet de la pénurie énergétique sur les systèmes d'élevage en France et ont montré que la prise en compte de cette pénurie implique de repositionner et de redistribuer l'élevage au sein des territoires.

Toutes les approches mentionnées ci-dessus ont en commun l'existence d'approches systémiques, construites sur la représentation d'éléments sélectionnés des systèmes agricoles. La construction de ces modèles permet de représenter la production agricole comme un système composé d'éléments qui interagissent pour donner naissance à un comportement émergent. Dans un monde où la sobriété dans l'utilisation des ressources et de l'énergie est requise, ces méthodes devraient être privilégiées pour guider la construction de modèles agricoles adéquats. L'élevage ne se limite pas à la production de viande ou de lait, mais englobe sa contribution à la biodiversité (Fraser et al., 2022), l'entretien des paysages ou son intégration dans les systèmes de production végétale, et au-delà (Martin et al., 2016). L'évaluation de la durabilité des systèmes de production animale va au-delà de l'efficacité animale pour la production de viande ou de lait, et doit également prendre en compte ces aspects.

6.1.5 Durabilité des méthodes de recherche employées

La recherche, comme toute autre activité humaine, doit réduire son empreinte environnementale, qui va au-delà des émissions de CO_2 (Steffen et al., 2015). Il est donc important pour les chercheurs de développer des méthodes scientifiques applicables dans un monde plus responsable. Lorsqu'on considère les activités ayant un impact négatif sur l'environnement, la recherche scientifique serait probablement l'une des dernières activités citées. À titre d'exemple, la recherche agricole s'est concentrée sur la réduction de l'empreinte des systèmes agricoles, mais l'empreinte de la recherche agricole elle-même est négligée. La quête de connaissances est souvent considérée comme une activité très « noble », qui ne devrait pas être entravée en raison de son énorme potentiel de transformation. À titre d'exemple, l'expérimentation animale en recherche biomédicale (Petetta et Ciccocioppo, 2021) ou les expériences sur le terrain avec des OGM (Kuntz, 2012) sont des sujets qui suscitent des conflits

entre la société et la communauté scientifique. La question de l'impact environnemental de la recherche est pour l'instant encore quasiment absente du débat public. Bien que quelques exemples aient récemment émergé, comme le débat sur la construction d'un accélérateur de particules par le CERN entre la Suisse et la France (Libération, février 2025), l'empreinte carbone des déplacements liés à la recherche a également été identifiée comme un fardeau majeur (Ben-Ari et al., 2024).

La question de savoir quelles recherches peuvent être menées tout en respectant l'exigence de durabilité doit être posée. En élevage, des recherches basées sur des résultats à l'échelle de la ferme, tels que la croissance animale, la production laitière ou la prévalence de maladies, pourraient encore être obtenues dans un monde plus sobre. Mais pour comprendre comment ces résultats sont construits, il est nécessaire de se plonger dans la biologie animale. La question serait alors de savoir comment mener des recherches en biologie et en physiologie avec un impact limité sur l'environnement (consommation d'énergie, consommation de plastique, émissions de CO_2, etc.). Il n'existe probablement pas encore de réponse évidente à cette question, et différentes pistes doivent être explorées.

Les recherches menées dans le cadre de cette thèse ont permis d'intensifier l'utilisation des résultats déjà publiés, grâce au développement du modèle mathématique. La réalisation d'expériences et la publication de résultats ont un coût environnemental, et leur intégration dans un modèle mathématique permet de les valoriser pleinement. De plus, les modèles mathématiques, grâce à la pensée systémique qu'ils offrent, peuvent servir d'outil pour guider la conception d'expériences futures. L'approche de culture cellulaire adoptée dans cette thèse s'est concentrée sur un nombre limité de mesures, principalement la composition isotopique par EA-IRMS. Pour de telles études exploratoires, il serait tentant de mesurer de nombreux paramètres afin de mieux appréhender le comportement du système cellulaire. Par exemple, on pourrait être tenté d'étudier la présence de transporteurs d'AA via l'expression des gènes (Bröer et Fairweather, 2018). Cependant, sans hypothèse préalable, ces résultats supplémentaires auraient ajouté du bruit à notre étude sans apporter d'informations supplémentaires. Il a donc été décidé d'exploiter pleinement les données de composition isotopique obtenues pour tirer des conclusions. Ensuite, sur la base des conclusions tirées de nos données et des nouvelles hypothèses formulées, d’autres expériences avec de nouvelles mesures pourraient être réalisées.

COLLEGE ECOLOGIE
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Titre : Approches *in silico* et *in vitro* pour investiguer le métabolisme des acides aminés dans l'intestin grêle

Mots clés : Nutrition, modèle mathématique, culture cellulaire, isotopes stables

Résumé : L'intestin grêle est le site d'une importante d'activité métabolique, avec un renouvellement quotidien de ses protéines. Il est supposé que l'activité métabolique de l'intestin grêle influence fortement l'apparition des acides aminés dans le sang pendant la digestion. L'objectif de cette thèse était de mieux comprendre le métabolisme intestinal des acides aminés pendant la digestion, en combinant deux approches alternatives à l'expérimentation animale. Tout d'abord, nous avons construit un modèle mathématique du métabolisme intestinal, pour un acide aminé générique. Ce modèle a été construit en représentant l'intestin grêle comme une série de segments, chacun conservant la même structure et représentant les principaux processus qui s'y déroulent. Ce modèle a ensuite été affiné et utilisé pour simuler différentes situations nutritionnelles. Il a été utilisé pour remettre en cause des concepts habituellement abordés *in vivo* tels que la digestibilité. En parallèle, nous avons développé une méthode pour étudier la cinétique d'utilisation des acides aminés par les entérocyes *in vitro*, en utilisant des cellules IPEC-J2 cultivées sur puits transwells. Après 14 jours de culture, de la [U-^{13}C ; ^{15}N]-glutamine ou lysine était insérée dans le milieu apical. Ensuite, pendant 4 heures à différents moments, le milieu (apical et basolatéral) et les cellules étaient récoltés et leur composition isotopique était analysée. Les résultats suggèrent une différence dans la cinétique d'absorption et d'accumulation dans les cellules entre ces deux acides aminés. La combinaison des approches de modélisation et de culture cellulaire offre un champ prometteur pour la recherche en nutrition et métabolisme.

Title : *In silico* and *in vitro* approaches to investigate small intestinal metabolism of amino acids

Keywords : Nutrition, mathematical modelling, cell culture, stable isotopes

Abstract: The small intestine is an important site of metabolic activity, representing 20% of whole-body energy expenditure, with a daily replacement of intestinal proteins. It is suspected that metabolic activity of the small intestine influences to a large extent appearance of amino acids in the blood during digestion. Studying fluxes of amino acids in the small intestine *in vivo* has been intended in pig, yet it has major ethical limitations. In this thesis, we sought to better understand the small intestinal metabolism of amino acids during digestion, by combining two approaches which are alternatives to animal experiments. First, we built a mathematical model of intestinal metabolism, for a generic amino acid. This model was built representing the small intestine as a series of segments, each retaining the same structure and representing the main processes ongoing. This model was then refined and used to simulate different nutritional situations. It was used to challenge concepts usually approached *in vivo* such as digestibility and first-pass metabolism. In parallel, we developed a method to study kinetic of use of amino acids by enterocytes *in vitro*, by using IPEC-J2 cells cultivated on transwells. After 14 days of culture, [U-^{13}C; ^{15}N]-glutamine or lysine was inserted in the apical medium. Then, during 4 hours at different time points, medium (apical and basolateral) and cells were harvested and analysed for their isotopic composition. Results suggest a difference in the kinetic of absorption and accumulation in cells in between these two amino acids. Combining mathemaical modelling and cell culture approaches offer a promising field for future research in the area of nutrition and metabolism.