



2021 ANIMAL NUTRITION
CONFERENCE OF CANADA



2021 COLLOQUE DE NUTRITION
ANIMALE DU CANADA

Proceedings

Cahier de conférences



Virtual Event
Événement virtuel

10-14
May/mai 2021

Feeding the Future: Precision Nutrition for Tomorrow's Animal

Alimenter l'avenir : Nutrition de précision
pour l'élevage de demain

Pre-conference symposium sponsor
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ANAC Welcome Message

Welcome to the 2021 Animal Nutrition Conference of Canada (ANCC)!

The Animal Nutrition Association of Canada is honoured to host this event and provide the animal feed industry with this exceptional learning and networking opportunity. Thanks to the dedication of the organizing committee, generosity of sponsors and amazing research shared from around the world, the ANCC truly has become a one-of-a-kind world class event.

This year marks the 5th conference since the ANCC was inaugurated in Quebec City and coupled with events of the past year, I find myself apt to compare the two. The global pandemic has shown us the importance of adapting to change, relying on science, adopting new technologies, and connecting with others to remain resilient through challenges. In many ways, these are all fundamental aspects of the conference as we look to the increasing challenges we face in the animal protein market.

One of the goals of the ANCC is to present the latest research and innovations in animal nutrition to help inform, inspire, and enhance the work of animal nutritionists. This year's program delivers on that objective. A conference of this magnitude takes an enormous amount of work and we would like to recognize all organizing committee members for their dedication to the success of ANCC 2021.

The ANCC would not be possible without the generous support of our industry partners. Thank you to the 2021 sponsors who have once again embraced the virtual conference format and enabled us to bring this conference to participants from around the world. We extend our appreciation as well to our dedicated speakers for sharing their expertise and students participating in the poster competition for giving us the opportunity to learn about their exciting research.

We hope you will take the opportunity to enjoy all the conference has to offer. Engage with colleagues and speakers using the interactive digital tools and connect with the new generation of feed industry professionals during the student poster competition and networking sessions.

Enjoy your learning and discussions at ANCC 2021 and I hope to see many of you at next year's conference in Saskatoon, Saskatchewan. Until then, take care and stay safe.

Melissa Dumont, agr.
Executive Director



We acknowledge that interpretation and translation was made possible with the financial support of the Government of Canada.



Message de bienvenue de l'ANAC

Bienvenue au Colloque de nutrition animale du Canada (CNAC) 2021 !

L'Association de nutrition animale du Canada a l'honneur de présenter cet événement et d'offrir à l'industrie de l'alimentation animale cette occasion exceptionnelle d'apprentissage et de réseautage. Grâce au dévouement du Comité organisateur, à la générosité des commanditaires et aux partages d'étonnantes recherches provenant de partout dans le monde, le CNAC est vraiment devenu un événement unique de classe mondiale.

Cette année marque le 5^e colloque depuis l'inauguration du CNAC à Québec et, compte tenu des événements de l'année écoulée, je suis portée à comparer les deux. La pandémie mondiale nous a montré l'importance de s'adapter au changement, de se fier à la science, d'adopter de nouvelles technologies et de se rapprocher des autres pour rester résilient face aux défis. À bien des égards, ce sont tous des aspects fondamentaux du colloque alors que nous nous dressons devant les défis croissants auxquels nous sommes confrontés sur le marché des protéines animales.

L'un des objectifs du CNAC est de présenter les dernières recherches et innovations en matière de nutrition animale afin d'informer, d'inspirer et d'améliorer le travail des nutritionnistes. Le programme de cette année répond à cet objectif. Un événement de cette ampleur exige énormément de travail et nous tenons à remercier tous les membres du Comité organisateur pour leur dévouement au succès du CNAC 2021.

Le CNAC ne serait pas possible sans le soutien généreux de nos partenaires industriels. Nous remercions les commanditaires de 2021 qui ont une fois de plus adopté le format du colloque virtuel et nous permettent ainsi d'offrir ce colloque à des participants du monde entier. Nous remercions également nos conférenciers d'avoir accepté de partager leur expertise et les étudiants qui ont participé au concours d'affiches de nous avoir donné l'occasion de découvrir leurs recherches passionnantes.

Nous espérons que vous saisissez l'occasion de profiter de tout ce que le colloque a à offrir. Communiquez avec vos collègues et les conférenciers à l'aide des outils numériques interactifs et rencontrez la nouvelle génération de professionnels de l'industrie de l'alimentation animale lors du concours d'affiches pour étudiants et des sessions de réseautage.

Profitez de votre apprentissage et de vos discussions au CNAC 2021 et j'espère vous voir nombreux au colloque de l'année prochaine à Saskatoon, en Saskatchewan. D'ici là, prenez soin de vous et restez en sécurité.

Melissa Dumont, agr.

Directrice exécutive

Canada 

Nous reconnaissons que l'interprétation et la traduction ont été rendues possibles grâce au soutien financier du gouvernement du Canada.

ANCC 2021 Organizing Committee / Comité organisateur du CNAC 2021

We are honoured to welcome you to the 5th annual Animal Nutrition Conference of Canada (ANCC). This year's conference remains online, marking the 2nd year we have brought you the ANCC in a virtual format. This year's digital conference environment will provide you with new ways to interact and network with conference participants, students and sponsors. The environment includes spaces for our sponsors and exhibitors to display their booths and an exhibit hall for our graduate students to display their research posters as part of a competition. We have also included an exciting gamification aspect, which we hope will encourage you to explore the platform to enhance your learning while accumulating chances to win prizes from our sponsors.

When deciding on the theme for our 2021 conference, the organizing committee reflected on the challenges that 2020 had presented to us. The agricultural industry weathered unprecedented upheaval in the face of the COVID-19 pandemic. The committee wanted to address the challenges the pandemic handed us while simultaneously looking to the future. With this goal in mind, the theme "Feeding the Future: Precision Nutrition for Tomorrow's Animal" was decided upon.

The organization committee has worked hard to bring you a robust program of talented researchers and speakers from across Canada and the globe, and we are excited to also be highlighting research from Canadian students and our industry partners. We are incredibly thankful to ANAC and our ANCC 2021 sponsors for their support in bringing you this high-quality program. We hope that this year's new format brings you exceptional value and that you will find the research presented both practical in its application and compelling in its implications for the future.

Stay healthy and safe and enjoy the conference!

Nous sommes fiers de vous accueillir au 5^e Colloque de nutrition animale du Canada (CNAC). Cette année, le colloque se déroulera en ligne, marquant la 2^e année que nous vous offrons le CNAC en format virtuel. L'encadrement numérique du colloque de cette année vous permettra de profiter de nouvelles façons d'interagir avec les participants, les étudiants et les commanditaires. Cet encadrement prévoit de l'espace pour que nos commanditaires et exposants puissent présenter leurs kiosques ainsi qu'un salon d'exposition où les étudiants afficheront leurs posters de recherche dans le cadre d'un concours. Nous avons aussi incorporé une facette de ludification qui, nous le souhaitons, vous encouragera à explorer la plateforme afin de rehausser votre expérience d'apprentissage, tout en accumulant des chances de gagner des prix fournis par nos commanditaires.

Au moment de déterminer le thème du colloque de 2021, le Comité organisateur s'est penché sur les défis de l'année 2020. L'industrie agricole a subi un bouleversement sans précédent face à la pandémie de COVID-19. Le comité voulait donc aborder les défis associés à la pandémie, tout en jetant un regard sur l'avenir. À la lumière de cet objectif, il a été décidé de retenir le thème « Alimenter l'avenir : Nutrition de précision pour l'élevage de demain ».

Le Comité organisateur a déployé tous les efforts pour vous offrir un programme rigoureux de chercheurs talentueux et de conférenciers canadiens et internationaux et nous sommes également emballés à l'idée de mettre en vedette la recherche d'étudiants canadiens et de nos partenaires de l'industrie. Nous remercions l'ANAC très sincèrement et nos commanditaires du CNAC 2021 pour leur soutien à vous présenter ce programme de très haute qualité. Nous espérons que le format de cette année aura une valeur exceptionnelle et que vous trouverez la recherche présentée à la fois pratique d'un point de vue application et convaincante quant à ses conséquences pour l'avenir.

Demeurez en santé, soyez prudent et bon colloque!

Holly McGill, Wallenstein Feed & Supply Ltd.
(Program Chair / Présidente du programme du colloque)

Rachel Gervais, Université Laval
(Academic Chair / Présidente de l'académie)

Bertrand Medina, Probiotech International Inc.
(Sponsorship Chair / Président du parrainage)

Renée Benczik, Wallenstein Feed & Supply Ltd.

Murray Feist, Saskatchewan Ministry of Agriculture

Jeff Keunen, Grand Valley Fortifiers

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Marie-Pierre Létourneau-Montminy, Université Laval

Leslie McKnight, Trouw Nutrition

Ilona Parenteau, Halchemix Canada

Renée Petri, Alltech Canada

Sharon Robinson, Halchemix Canada

Gemunu Widyaratne, Proveta Nutrition Ltd.

Sarah Hopkins, Josée Lafontaine & Nancy Stonos-Smith
ANAC

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2021 ANCC CONFERENCE PROGRAM

PROGRAMME DU COLLOQUE CNAC 2021

Virtual Conference - May 10 - 14, 2021
Conférence virtuelle - Les 10 - 14 mai 2021

Feeding the Future: Precision Nutrition for Tomorrow's Animal *Alimenter l'avenir : Nutrition de précision pour l'élevage de demain*

Virtual exhibit halls and networking opportunities are available throughout the conference.
Des salles d'exposition virtuelles et des possibilités de mise en réseau sont disponibles tout au long de la conférence.

Pre-Conference Symposium *Symposium pré-conférence*

Monday, May 10
Le lundi 10 mai



Precision Intestinal Nutrition *Nutrition intestinale de précision*

12:00 EDT/HAE	Welcome / <i>Bienvenue</i>	Dr. Elizabeth Santin Jefo Nutrition
12:05 EDT/HAE	The symbiosis between nutrition, intestinal microbiota and host immunity for animal health and production <i>Symbiose entre nutrition, microbiote intestinal et immunité de l'hôte au profit de la santé et de la production animales</i>	Dr. Mike Kogut USDA
12:50 EDT/HAE	Vitamins as co-factors and antioxidants to boost immunity and gut health <i>Vitamines utilisées comme cofacteurs et antioxydants pour stimuler l'immunité et la santé du tube digestif</i>	Dr. Doug Korver University of Alberta
1:35 EDT/HAE	Health break / <i>Pause-santé</i>	
1:50 EDT/HAE	How the maternal microbiota affects milk quality and progeny health and production: Can we modulate it by nutrition? <i>Influence du microbiote maternel sur la qualité du lait et sur la santé et la production des descendants</i>	Dr. Rodrigo Carvalho Bicalho Cornell University
2:35 EDT/HAE	The immunometabolic interface between host and microbiota <i>L'interface immunométabolique entre hôte et microbiote</i>	Dr. Ryan Arsenault University of Delaware
3:20 EDT/HAE	Round table with all pre-conference speakers <i>Table ronde réunissant tous les intervenants du symposium pré-conférence</i>	Chaired by / <i>Présidée par</i> Dr. Elizabeth Santin Jefo Nutrition

All sessions will be presented in English or French with the option for simultaneous interpretation.
Toutes les sessions seront présentées en anglais ou en français, avec possibilité d'interprétation simultanée.



2021 ANCC CONFERENCE PROGRAM

PROGRAMME DU COLLOQUE CNAC 2021

Tuesday, May 11th / Le mardi 11 mai

Opening Plenary Speakers / <i>Plénière d'ouverture</i>		
12:00 EDT/HAE	Opening remarks from ANAC & Organizing Committee welcome <i>Propos d'ouverture de l'ANAC & Bienvenue du comité organisateur</i>	
12:15 EDT/HAE	The role of mechanistic models in precision dairy nutrition and on-farm decision support <i>Le rôle des modèles mécanistes en nutrition laitière de précision et comme outils d'aide aux décisions à la ferme</i>	Dr. Jennifer Ellis University of Guelph
1:00 EDT/HAE	Identification and validation of biomarkers of enteric methanogenesis in cattle for use in methane mitigation strategies <i>Identification et validation de biomarqueurs de la méthanogénèse entérique chez les bovins aux fins de leur utilisation dans les stratégies d'atténuation du méthane</i>	Dr. Cécile Martin French National Research Institute for Agriculture, Food and the Environment (INRAE)
1:45 EDT/HAE	Health break / <i>Pause-santé</i>	
2:00 EDT/HAE	Lighting programs for broilers : Can they change broiler feeding patterns and feed utilization? <i>Les programmes d'éclairage pour les poulets à griller : peuvent-ils influencer sur les comportements alimentaires et l'efficacité alimentaire</i>	Dr. Karen Schwan-Lardner University of Saskatchewan
2:45 EDT/HAE	Zinc replacement strategies for preventing piglet diarrhoea <i>Stratégies de substitution du zinc dans la prévention de la diarrhée chez le porcelet</i>	Dr. Charlotte Lauridsen Aarhus University
3:30 EDT/HAE	End of live sessions. Take this opportunity to visit the exhibit halls. <i>Fin des sessions en direct. Profitez de l'occasion pour visiter les salles d'exposition.</i>	

NEW - Student Networking Session / NOUVEAU - Événement de réseautage pour les étudiants

Join the **career panel discussion** at 4:00 EDT,
followed by **breakout sessions** with perspective feed industry employers until 5:30 EDT.

Joignez-vous à **la table ronde sur les carrières** à 4:00 HAE,
suivit de **séances en petits groupes** avec les employeurs potentiels de l'industrie de l'alimentation animale jusqu'à 5:30 HAE.

All sessions will be presented in English or French with the option for simultaneous interpretation.
Toutes les sessions seront présentées en anglais ou en français, avec possibilité d'interprétation simultanée.



2021 ANCC CONFERENCE PROGRAM

PROGRAMME DU COLLOQUE CNAC 2021

Wednesday, May 12th / Le mercredi 12 mai

Monogastric Speakers / Séance sur les monogastriques		
12:00 EDT/HAE	Jean F. Bernier Lectureship: Basic elements, difficulties and pitfalls on the development and application of precision nutrition techniques for smart pig farming Conférence en l'honneur de Jean F. Bernier : <i>Éléments fondamentaux, difficultés et pièges du développement et de l'application de techniques de nutrition de précision en production porcine intelligente</i>	Dr. Candido Pomar Agriculture and Agri-Food Canada
1:00 EDT/HAE	Bird-e – the poultry e-feeding system: basis and applications <i>Bird-e – Le système d'alimentation électronique de volaille : fondements et applications</i>	Dr. Eva Pampouille French Poultry Technical Institute (ITAVI)
1:45 EDT/HAE	Health break / Pause-santé	
Ruminant Speakers / Séance sur les ruminants		
2:00 EDT/HAE	Meeting B vitamin requirements of dairy cows, an effective way to improve metabolic efficiency <i>Comblent les besoins en vitamines B de la vache laitière, un moyen efficace d'améliorer l'efficacité métabolique</i>	Dr. Christiane Girard Agriculture and Agri-Food Canada
2:45 EDT/HAE	How the cow's daily pattern of feed intake impacts milk synthesis <i>Synchronisation de la consommation alimentaire pour la synthèse du lait</i>	Dr. Kevin Harvatine Pennsylvania State University

Graduate Student Poster Competition / Concours d'affiches pour étudiants diplômés

Visit the Student Exhibit Hall to see what the next generation of scientists is bringing to our industry.

Students will be present from 3:30 to 4:30 EDT to discuss their research and answer your questions.

Visitez la salle d'exposition des étudiants pour voir ce que la prochaine génération de scientifiques apporte à notre industrie.

Les étudiants seront présents de 3 :30 à 4:30 HAE pour discuter de leurs recherches et répondre à vos questions.

2021 ANCC CONFERENCE PROGRAM

PROGRAMME DU COLLOQUE CNAC 2021

Thursday, May 13th / Le jeudi 13 mai

Graduate Student Poster Competition / Concours d'affiches pour étudiants diplômés

Visit the Student Exhibit Hall to see what the next generation of scientists is bringing to our industry.

Students will be present from 11:00 to 12:00 EDT to discuss their research and answer your questions.

Visitez la salle d'exposition des étudiants pour voir ce que la prochaine génération de scientifiques apporte à notre industrie.

Les étudiants seront présents de 11:00 à 12:00 HAE pour discuter de leurs recherches et répondre à vos questions.

Ruminant Speakers / Séance sur les ruminants		
12:00 EDT/HAE	Maximizing the feed value of high fibre forages to ruminants: The impact of ammonia fibre expansion technology (AFEX) and novel recombinant fibrolytic enzymes on digestion and the rumen microbial population <i>Maximiser la valeur alimentaire des fourrages riches en fibres pour les ruminants : l'impact du procédé d'expansion des fibres à l'ammoniaque (AFEX) et des nouvelles enzymes fibrolytiques recombinantes sur la digestion et la population microbienne du rumen</i>	Dr. Gabriel Ribeiro University of Saskatchewan
12:45 EDT/HAE	Precision determination of energy and protein requirements of grazing and feedlot animals <i>Détermination avec précision des besoins en énergie et en protéine des animaux au pâturage et en parc d'engraissement</i>	Dr. Luis Tedeschi Texas A&M University
1:30 EDT/HAE	Health break / Pause-santé	
Monogastric Speakers / Séance sur les monogastriques		
1:45 EDT/HAE	Precision feeding of poultry: Matching nutrient supply with the nutrient requirements of individual birds <i>Alimentation de précision en aviculture : assurer la correspondance entre l'apport en nutriments et les besoins nutritifs individuels des oiseaux</i>	Dr. Martin Zuidhof University of Alberta
2:30 EDT/HAE	Contributions of enzyme technology to non-ruminant nutrition <i>Contributions de la technologie des enzymes à la nutrition des monogastriques</i>	Dr. Olayiwola Adeola Purdue University
3:15 EDT/HAE	End of live sessions. Take this opportunity to visit the exhibit halls. <i>Fin des sessions en direct. Profitez de l'occasion pour visiter les salles d'exposition.</i>	

All sessions will be presented in English or French with the option for simultaneous interpretation.

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2021 ANCC CONFERENCE PROGRAM

PROGRAMME DU COLLOQUE CNAC 2021

Friday, May 14th / Le vendredi 14 mai

Graduate Student & Closing Plenary Speakers / <i>Les étudiants diplômés & plénière de clôture</i>		
12:00 EDT/HAE	Announcement of Graduate Student Poster Competition winners <i>Annonce des gagnants du concours d'affiches des étudiants diplômés</i>	
12:05 EDT/HAE	ANAC Scholarship Recipient: Yeast (<i>Saccharomyces cerevisiae</i>) as a feed additive for feedlot cattle Récipiendaire de la bourse d'études de l'ANAC : La levure (<i>Saccharomyces cerevisiae</i>) utilisée comme additif dans l'alimentation des bovins en parc d'engraissement	Melissa Williams University of Guelph Graduate Student / Étudiante diplômée
12:15 EDT/HAE	The effects of supplementing low-complexity deoxynivalenol-contaminated nursery diets with NutraMix™ or fish oil on growth performance and immune function <i>Effets de la supplémentation avec NutraMix^{MC} ou de l'huile de poisson des rations de pouponnière peu complexes contaminées par le désoxynivalénol sur la performance de croissance et l'immunité</i>	Élise Lafleur Larivière University of Guelph Graduate Student / Étudiante diplômée
12:25 EDT/HAE	Maternal metabolizable protein and rumen-protected methionine impacts gene expression in muscle <i>Influence des protéines maternelles métabolisables et de la méthionine protégée sur l'expression génétique dans les muscles</i>	Kortney Acton University of Guelph Graduate Student / Étudiante diplômée
12:40 EDT/HAE	Q&A with graduate student speakers <i>Séance de questions et réponses avec les étudiantes diplômées</i>	
1:00 EDT/HAE	Using automated technology to achieve precision in dairy nutrition <i>Usage de la technologie pour atteindre la précision en nutrition laitière</i>	Dr. Trevor DeVries University of Guelph
1:45 EDT/HAE	Health break / Pause-santé	
2:00 EDT/HAE	Investigating behavioural and brain responses as well as gut-brain signals to understand the relationships between nutrition and health in the pig model <i>Étudier les réponses comportementales et cérébrales ainsi que l'axe de communication intestin-cerveau pour comprendre les liens entre la nutrition et la santé dans le modèle porcin</i>	Dr. David Val-Laillet French National Research Institute for Agriculture, Food and the Environment (INRAE)
2:45 EDT/HAE	Closing remarks / <i>Propos de clôture</i>	

All sessions will be presented in English or French with the option for simultaneous interpretation.
Toutes les sessions seront présentées en anglais ou en français, avec possibilité d'interprétation simultanée.



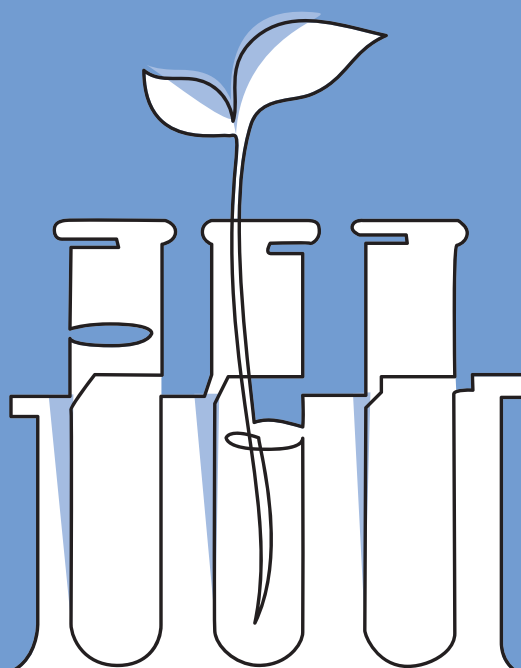
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The Immunometabolic Interface Between Host and Microbiota

L'interface immunométabolique entre hôte et microbiote

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Abstract

Immunometabolism is the study of immunity and metabolism as an integrated system. The prototypical organ for such study is the gut. Central to the proper and optimum functioning of the gut, thus its immunometabolism, is the microbiota. Often the microbiota is considered only in the context of what functions, molecules and signals it provides to the host. However, the interactions between the host and microbiota must fundamentally be understood as bidirectional communication. In the realm of metabolism, it is clear that the microbiota provides critical metabolites to the host, for example through bacterial fermentation of indigestible feed components. The host also provides a nutrient source for the bacteria of the gut, an example being glycans in mucus feeding mucosal-associated commensal bacteria. In the realm of immunity, significant research has been conducted on how microbiota signals aid in proper immune development. An early microbial colonization from the mother can aid her offspring immunologically throughout life. The immune system also dictates which microbes may reside on mucosal surfaces. Only in understanding all of these communication pathways, can we start to uncover the complex host-microbiota interface, and start to critically evaluate intervention strategies in context. Tradeoffs must also be understood in animal production, for example, a diverse microbiota can benefit host immunity and disease resistance, but may reduce optimal feed fermentation. When considering nutrition, feed additives and antibiotic alternatives we must study both the indirect and, critically, direct impacts of both metabolism and immunity on both host and microbiota simultaneously.

Résumé

L'immunométabolisme est l'étude de l'immunité et du métabolisme en tant que système intégré. L'organe prototype de cette discipline est l'intestin. Le microbiote est au coeur du fonctionnement normal et optimal de l'intestin, donc de son immunométabolisme. Souvent, le microbiote n'est considéré que pour les fonctions, molécules et signaux qu'il procure à l'hôte. Cependant, les interactions entre l'hôte et le microbiote doivent fondamentalement être interprétées comme une communication bidirectionnelle. Dans le contexte du métabolisme, il est clair que le microbiote fournit des métabolites essentiels à l'hôte, notamment par la fermentation bactérienne de composants alimentaires non digestibles. L'hôte fournit également une source de nutriments aux bactéries intestinales, par exemple les glycanes du mucus qui nourrissent les

bactéries commensales associées aux muqueuses. Dans le domaine de l'immunité, des recherches importantes ont été menées sur la manière dont les signaux du microbiote aident au bon développement du système immunitaire. Une colonisation microbienne précoce de la mère peut aider sa progéniture sur le plan immunologique toute sa vie durant. Le système immunitaire dicte également quels microorganismes peuvent résider sur les surfaces des muqueuses. Ce n'est qu'en faisant la lumière sur toutes ces voies de communication que nous pourrons commencer à expliquer l'interface complexe entre l'hôte et son microbiote et à évaluer de manière critique les stratégies d'intervention dans leur contexte. Les compromis à faire pour la production animale doivent également être mesurés : par exemple, un microbiote diversifié peut contribuer à l'immunité de l'hôte et à la résistance aux maladies, mais peut en même temps nuire à la fermentation optimale des aliments. Lorsque nous considérons la nutrition, les additifs alimentaires et les solutions de remplacement aux antibiotiques, nous devons étudier simultanément les impacts indirects et, de manière critique, directs du métabolisme et de l'immunité sur l'hôte et son microbiote.

How the Maternal Microbiota Affects Milk Quality and Progeny Health and Production: Can we Modulate it by Nutrition?

Influence du microbiote maternel sur la qualité du lait et sur la santé et la production des descendants

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Abstract

Since the discovery and elucidation of deoxyribonucleic acid (DNA) and its structure in the 1950s by Watson and colleagues (Watson and Crick, 1953, Feughelman et al., 1955, Wilkins, 1956) strategies and multiple technological innovations have been made in efforts to better understand the complexity and diversity of genomes in a variety of ecosystems, including those in the health and disease. The term metagenome was first described by Jo Handelsman in 1998 as “the genomes of the total microbiota found in nature” (Handelsman et al., 1998). More recently, with the development and application of high throughput sequencing methods in combination with the growing recognition of the importance of the microbial community a National Science and Technology Council Committee of US government scientists was created in 2015 (Stulberg et al., 2016). The US government scientists defined microbiome as “a multi-species community of microorganisms in a specific environment (that is, host, habitat or ecosystem)” and microbiome research as “those studies that emphasize community-level analyses with data derived from genome-enabled technologies” (Stulberg et al., 2016).

Mounting evidence on microbiome and its impact on host health and metabolic functions has been generated by next-generation sequencing over the last few years. Remarkably, the microbiome is now referred to as the “new biomarker” of health (Shukla et al., 2017) mainly due to its role in maintaining host physiology (Sommer and Bäckhed, 2013, Barrett and Wu, 2017), maturation and “education” of the immune system (Kelly et al., 2007, Chung et al., 2012) and its potential to mediate host metabolic development (Cho et al., 2012, Cox et al., 2014). Since coevolution of mammals and their microbiota has occurred over millions of years (Ley et al., 2008) is not a surprise that this tight connection between host and its microbiota exists.

In veterinary medicine, symbiosis between ruminant host and microbial population is essential for animal survival and existence, mainly due to its role in converting plant and grain materials, consumed by the host, into available energy resources that are subsequently absorbed and metabolized by the host (Hungate, 1966). Given its complexity and importance to the ruminant existence, the bovine gastrointestinal tract microbiota has been extensively investigated (Khafipour et al., 2009, Jami and Mizrahi, 2012, Jami et al., 2013, Meale et al., 2016, Dill-McFarland et al., 2017). However, it is becoming increasingly apparent that microbial communities in other anatomical sites, such as mammary gland (Oikonomou et al., 2012,

Oikonomou et al., 2014, Addis et al., 2016) and airways (Holman et al., 2017), are also relevant for bovine health.

Résumé

Depuis la découverte et l'élucidation de l'acide désoxyribonucléique (ADN) et de sa structure dans les années 1950 par Watson et ses collègues (Watson et Crick, 1953, Feughelman *et al.*, 1955, Wilkins, 1956), des stratégies et de nombreuses innovations technologiques ont été mises en œuvre pour mieux comprendre la complexité et la diversité des génomes dans divers écosystèmes, y compris celui de la santé et des maladies. Le terme *métagénome* a été initialement utilisé par Jo Handelsman en 1998 pour représenter « les génomes de l'ensemble du microbiote présent dans la nature » (Handelsman *et al.*, 1998). Plus récemment, compte tenu du développement et de l'utilisation de méthodes de séquençage à haut débit et de l'importance de plus en plus grande accordée à la communauté microbienne, un comité du conseil national des sciences et des technologies composé de scientifiques du gouvernement américain a été créé en 2015 (Stulberg *et al.*, 2016). Les scientifiques du gouvernement américain ont défini le microbiome comme « une communauté multi-espèces de microorganismes dans un environnement spécifique (c'est-à-dire un hôte, un habitat ou un écosystème) » et la recherche sur le microbiome comme « les études qui mettent l'accent sur les analyses à l'échelle de la communauté à partir de données issues des technologies génomiques » (Stulberg *et al.*, 2016).

Au cours des dernières années, le séquençage de nouvelle génération a permis d'accumuler des preuves sur le microbiome et son impact sur la santé de l'hôte et les fonctions métaboliques. Il est remarquable que le microbiome soit désormais considéré comme le « nouveau biomarqueur » de la santé (Shukla *et al.*, 2017), principalement en raison de son rôle dans le maintien de la physiologie de l'hôte (Sommer et Bäckhed, 2013, Barrett et Wu, 2017), la maturation et l'« éducation » du système immunitaire (Kelly *et al.*, 2007, Chung *et al.*, 2012) et son aptitude potentielle à servir de médiateur du développement métabolique de l'hôte (Cho *et al.*, 2012, Cox *et al.*, 2014). Puisque la coévolution des mammifères et de leur microbiote s'est opérée sur des millions d'années (Ley *et al.*, 2008), l'existence de ce lien étroit entre l'hôte et son microbiote n'a rien de surprenant.

Pour la médecine vétérinaire, la symbiose entre l'hôte ruminant et la population microbienne est essentielle à la survie et à l'existence des animaux, principalement en raison de son rôle dans la conversion des matières végétales et céréalières consommées en ressources énergétiques disponibles qui sont ensuite absorbées et métabolisées par l'hôte (Hungate, 1966). Considérant sa complexité et son importance pour l'existence des ruminants, le microbiote du tube digestif bovin a fait l'objet d'études approfondies (Khafipour *et al.*, 2009, Jami et Mizrahi, 2012, Jami *et al.*, 2013, Meale *et al.*, 2016, Dill-McFarland *et al.*, 2017). Toutefois, il devient de plus en plus évident que les communautés microbiennes d'autres sites anatomiques, tels que la glande mammaire (Oikonomou *et al.*, 2012, Oikonomou *et al.*, 2014, Addis *et al.*, 2016) et les voies respiratoires (Holman *et al.*, 2017), jouent également un rôle dans la santé bovine.

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The Symbiosis Between Nutrition, Intestinal Microbiota and Host Immunity for Animal Health and Production

Symbiose entre nutrition, microbiote intestinal et immunité de l'hôte au profit de la santé et de la production animales

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Abstract

Within an animal's interactive physiological networks, the complexity of nutritional interactions is not solely confronted by the host but is made substantially greater since the animal plays host to entire communities of commensal and symbiotic microbes that derive nutrients from the host diet and provide vital nutrients to the host. The gut microbiome is involved in the development, function, and maturation of the host immune cells locally and systemically. Under the influence of diet, the composition of the gut microbiome as well as the commensal-derived nutrients and metabolites are altered. These diet-induced shifts in microbiome composition and commensal-derived nutrients and metabolites have profound direct effects on host immunity. Until recently, the interaction between nutrition/diet host immunity has virtually ignored the effect of the gut microbiome. Many studies have concluded a direct effect of a specific nutrient on the immune system without determining the effect on the gut microbiome. Therefore, knowing that diet and commensal microbiota interaction has a remarkable influence on the host immune response, many studies indicating poultry diets and their components directly affect the avian immune response may have to be re-evaluated, since the role of the gut microbiota was not considered. Although much is still to be learned about the microorganisms that comprise the avian gut microbiota, it is paramount that avian nutritionists and immunologists consider the role of the gut microbiota when investigating the role of the diet and dietary components on avian immune function and their effects on health and production.

Résumé

Au sein des réseaux physiologiques interactifs d'un animal, la complexité des interactions nutritionnelles est considérablement accrue du fait que l'hôte héberge des communautés entières de microorganismes commensaux et symbiotiques qui tirent leurs nutriments des aliments de l'hôte et lui fournissent des nutriments vitaux. Le microbiome intestinal intervient dans le développement, le fonctionnement et la maturation des cellules immunitaires de l'hôte, à l'échelle locale et systémique. Sous l'influence du régime alimentaire, la composition du microbiome intestinal ainsi que les nutriments et métabolites associés aux organismes commensaux sont modifiés. Ces modifications induites par l'alimentation ont des répercussions directes importantes sur le système immunitaire de l'hôte. Jusqu'à récemment, l'interaction entre la

nutrition et l'immunité de l'hôte ne tenait pratiquement pas compte de l'effet du microbiome intestinal. De nombreuses études ont conclu à un effet direct d'un nutriment spécifique sur le système immunitaire sans en déterminer l'effet sur le microbiome intestinal. Par conséquent, puisque l'interaction entre le régime alimentaire et le microbiote commensal a une influence notable sur la réponse immunitaire de l'hôte, de nombreuses études montrant que le régime alimentaire des volailles et ses composants influencent directement la réponse immunitaire des oiseaux pourraient devoir être réévaluées, le rôle du microbiote intestinal n'ayant pas été considéré. Bien qu'il reste beaucoup à apprendre sur les microorganismes qui composent le microbiote intestinal des oiseaux, il est primordial que les experts de la nutrition et de l'immunologie aviaires tiennent compte du rôle du microbiote intestinal lorsqu'ils étudient le rôle de la ration et des éléments qui la composent sur la fonction immunitaire des oiseaux et leurs effets sur la santé et la production.

The Role of Vitamins as Co-factors and Antioxidants in Immunity and Gut Health

Vitamines utilisées comme cofacteurs et antioxydants pour stimuler l'immunité et la santé du tube digestif

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Abstract

The importance of vitamin nutrition in poultry feeding has been recognized for decades, yet precise vitamin requirements for modern, high producing have not been investigated. Because of their low inclusion rates, vitamins have a relatively small impact on the cost of feed. Many vitamins lose activity over time or under high-temperature feed processing. Vitamins, especially the water-soluble vitamins, are tolerated at many times the established minimum recommended levels. Finally, the standard minimum recommendations published in sources such as the 1994 Nutrient Requirements of Chickens (NRC) are based largely on achieving maximum growth or egg production, rather than the optimization of product yield, quality, or bird health. Therefore commercial nutritionists generally including large safety margins for each vitamin.

Most vitamin requirement studies in poultry were completed before the 1980's; few classical vitamin requirement studies have been conducted since then. Major advances in efficiency of meat and egg production have changed the context for vitamin nutrition. Increased production efficiency without concomitant increases in vitamin supplementation implies an actual reduction in vitamin intake relative per unit of product produced. The global move towards antibiotic-free poultry meat production, and extended laying cycles for layers increases the importance of the immune system, and gut health in particular. The role of vitamins with particular impacts on immunity and gut integrity (specifically vitamins A, D and E) will be reviewed in the context of opportunities in the face of the challenges of modern poultry production.

Résumé

L'importance des vitamines dans l'alimentation des volailles est reconnue depuis des décennies, mais les besoins précis en vitamines des oiseaux modernes et à rendement élevé n'ont pas été étudiés. En raison de leur faible taux d'incorporation, les vitamines pèsent relativement peu sur le coût des aliments. L'activité de nombreuses vitamines diminue avec le temps ou pendant la transformation à haute température des aliments. Les vitamines, en particulier les vitamines

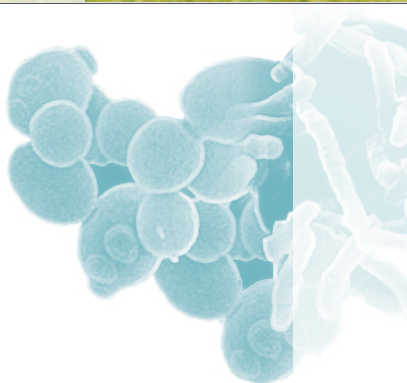
hydrosolubles, sont tolérées à des doses souvent plusieurs fois supérieures aux concentrations minimales recommandées. Enfin, les recommandations minimales standard publiées dans des sources telles que le guide des besoins nutritionnels des poulets (NRC) de 1994 ont généralement pour objectif la maximisation de la croissance ou de la production d'œufs, plutôt que l'optimisation du rendement, de la qualité ou de la santé des oiseaux. C'est pourquoi les experts en nutrition utilisent généralement de grandes marges de sécurité pour chaque vitamine.

La plupart des études sur les besoins en vitamines des volailles ont été réalisées avant les années 1980, et peu d'études classiques sur le sujet ont été menées depuis ce temps. Des progrès majeurs en matière d'efficacité de la production de viande et d'œufs ont modifié le contexte de l'utilisation des vitamines en nutrition. Une efficacité de production accrue sans augmentation concomitante de la supplémentation en vitamines suppose une réduction réelle de la consommation de vitamines par unité de produit fabriqué. L'évolution mondiale vers une production de viande de volaille sans antibiotiques et vers des cycles de ponte prolongés pour les pondeuses accroît l'importance du système immunitaire et de la santé intestinale en particulier. Le rôle des vitamines ayant un impact spécifique sur l'immunité et sur l'intégrité intestinale (surtout les vitamines A, D et E) sera examiné dans le contexte des possibilités offertes compte tenu des défis que pose la production moderne de volailles.

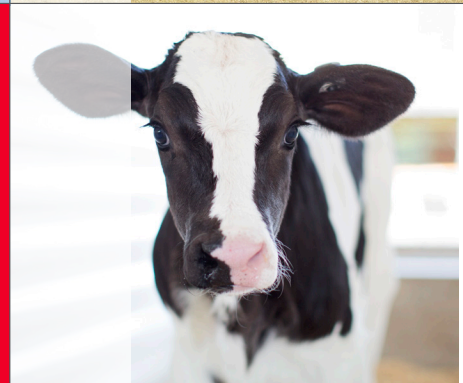
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Maternal Metabolizable Protein and Rumen-Protected Methionine Supplementation Impacts Steer Gene Expression in Muscle

Influence des protéines maternelles métabolisables et de la méthionine protégée sur l'expression génétique dans les muscles

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Abstract

Maternal nutrition can influence fetal growth in utero and progeny development throughout life. To assess how maternal nutrition during late gestation impacts mRNA expression of genes related to growth, myogenesis, and adipogenesis in steers, 138 Angus cows were used in a 3x2 factorial arrangement of treatments (Exp. 1). For the last 8 wks of gestation, cows were randomly assigned to be fed at 90, 100, or 110% of metabolizable protein (**MP**) requirements, with(without) 9g/d of rumen-protected methionine (**RPM**). In addition, to assess similar outcomes in an industry applied setting, a 2nd study of 67 Angus cows had unlimited access to water and hay and received 0.75kg/hd/d of supplemental pellet supplying 12g/d of rumen-protected methionine (**MET**), or an identical pellet without additional methionine (**CON**; Exp. 2). After weaning, steer progeny ($n_1=51$, $n_2=34$) were assigned to pens by weight and fed a grower diet (58% corn silage/26% haylage/15% SBM) for 47d, followed by a finisher diet (78% high moisture corn/12% haylage/8% SBM) for 115 ± 31.5 d until slaughter. Muscle samples were collected for mRNA analysis. Data analyzed using PROC GLIMMIX in SAS, with maternal treatments as fixed effects and pen as a random effect. In Exp. 1, myogenin expression in muscle (*MYOG*) was greater (90%: 0.72, 100%: 0.94, 110%: 1.00; SEM=0.083, $P=0.02$) in steers from cows fed 110% MP requirements. Pyruvate kinase (*PKM*) expression (associated with lean tissue growth) was reduced (RPM: 0.81, NoRPM: 1.16; SEM=0.092, $P=0.02$) in steers from RPM supplemented cows. In Exp. 2, MET supplementation increased *PKM* expression (MET: 1.98, CON: 1.35; SEM=0.221, $P=0.05$) and myoblast determination protein-1 (*MYOD*; MET: 1.82, CON: 1.20; SEM=0.190, $P=0.02$) in muscle. These data suggest that maternal nutrition in late gestation influences offspring muscle development throughout life, which could influence carcass grade and muscle characteristics.

Key words: developmental programming, methionine, metabolizable protein, maternal nutrition, myogenin

Résumé

La nutrition de la mère peut influencer la croissance du fœtus in utero et le développement de la progéniture tout au long de sa vie. Pour évaluer l'impact de la nutrition de la mère en fin de gestation sur l'expression de l'ARNm des gènes liés à la croissance, à la myogenèse et à

l'adipogenèse chez les bouvillons, 138 vaches Angus ont été utilisées dans une disposition factorielle 3x2 de traitements (Exp. 1). Pendant les 8 dernières semaines de gestation, les vaches ont été assignées au hasard à une ration couvrant 90 %, 100 % ou 110 % des besoins en protéines métabolisables (**MP**), avec (sans) 9 g/j de méthionine protégée (**RPM**). De plus, afin d'évaluer des résultats similaires dans un contexte industriel, une deuxième étude portant sur 67 vaches Angus profitant d'un accès illimité à l'eau et au foin et recevant 0,75 kg/tête/j d'un complément en granulés fournissant 12 g/j de méthionine protégée contre la dégradation dans le rumen (**MET**), ou d'un complément en granulés identique sans méthionine supplémentaire (**CON**; Exp. 2). Après le sevrage, les bouvillons de ces vaches ($n_1=51$, $n_2=34$) ont été répartis dans des enclos en fonction de leur poids et ont reçu une ration de croissance (58 % d'ensilage de maïs / 26 % d'ensilage préfané / 15 % de tourteau de soya) pendant 47 jours, puis une ration de finition (78 % de maïs humide / 12 % d'ensilage préfané / 8 % de tourteau de soya) pendant $115 \pm 31,5$ jours, jusqu'à l'abattage. Des échantillons de muscles ont été prélevés pour fins d'analyse de l'ARNm. Les données ont été analysées par PROC GLIMMIX en SAS, considérant les traitements maternels comme effets fixes et l'enclos comme effet aléatoire. Dans l'Exp. 1, l'expression de la myogénine dans les muscles (*MYOG*) a augmenté (90 % : 0,72, 100 % : 0,94, 110 % : 1,00; erreur-type=0,083, $P=0,02$) chez les bouvillons issus de vaches nourries à 110 % des besoins en MP. L'expression de la pyruvate-kinase (PKM) (associée à la croissance du tissu maigre) a diminué (RPM : 0,81, sans RPM : 1,16; erreur-type=0,092, $P=0,02$) chez les bouvillons nés de vaches supplémentées en méthionine protégée. Dans l'Exp. 2, la supplémentation en méthionine protégée a augmenté l'expression de la PKM (MET : 1,98, CON : 1,35; erreur-type=0,221, $P=0,05$) et la protéine-1 de détermination des myoblastes (MYOD; MET : 1,82, CON : 1,20; erreur-type=0,190, $P=0,02$) dans le muscle. Ces données suggèrent que la nutrition maternelle en fin de gestation influence le développement musculaire de la progéniture tout au long de sa vie, ce qui pourrait avoir une incidence sur le classement de la carcasse et les caractéristiques des muscles.

Introduction

Maternal nutrition during gestation and early life events impact offspring development throughout life, in a phenomenon known as developmental programming. The Barker hypothesis explains that undernutrition during specific developmental periods in pregnancy can create a scenario of pre- and post-partum metabolic mismatch (Hales and Barker, 2001). Metabolic mismatch occurs when offspring of undernourished cows are 'programed' to expect limited nutritional resources pre-partum, but postnatally consume diets that meet or exceeded nutritional requirements, which can influence fat and muscle development (Godfrey et al., 2007). In late gestation, fetal growth exponentially increases (Prior and Laster, 1979), additionally, offspring muscle fibres are maturing at this time, and intramuscular adipocytes (which accumulate fat throughout life and leads to marbling) begin to form (Du et al., 2010). Therefore, dietary manipulations specifically during late gestation may have a lasting impact on offspring development and meat quality. Previous studies show that maternal crude protein supplementation in late gestation influences steer offspring performance in the feedlot and carcass quality (Stalker et al., 2007; Larson et al., 2009). However, minimal research has been conducted to assess how maternal metabolizable protein (MP) alone influences steer offspring throughout life. Hare et al. (2019) observed that feeding cows 133% MP requirements did not impact gene expression (insulin-like growth factors (*IGFs*), insulin receptor (INSR), myogenic

differentiation (*MYOD*), myogenin (*MYOG*)) in muscle from heifer calves at weaning. However, further examination is required to determine how maternal MP level influences steers in the feedlot. Methionine is an essential amino acid and is involved in many metabolic pathways in the body (Jacometo et al., 2017) and supplementation of methionine in maternal diets can alter offspring gene expression (Jacometo et al., 2017; Zhang, 2018). However, the majority of research investigating the impact of methionine on offspring have only followed dairy (Batistel et al., 2017; Alharthi et al., 2018) and beef (Clements et al., 2017; Moriel et al., 2020) calves from birth until weaning at 8 weeks and 6 months of age, respectively. Therefore, research to determine how maternal methionine supplementation impacts offspring development past weaning is warranted. The main objectives of this study were to assess how maternal metabolizable protein concentration and rumen-protected methionine supplementation in late gestation impacts beef steer offspring development in the feedlot, and carcass quality. To gain further insight into potential mechanisms involved in offspring development, the expression of genes associated with growth, muscle development, and fat accumulation were assessed.

Methods

In a research-intensive study (Exp. 1), 138 Angus cross cows were randomly assigned to one of three protein treatments, 90, 100, or 110% of metabolizable protein (**MP**) requirements. Half of each group was received pellets containing nine g/d of rumen-protected methionine (**RPM**, Smartamine[®]M), or no additional pellets (**NoRPM**). These diets were formulated to be isocaloric through the addition of palm fat (cow performance data previously reported by Collins et al., 2019). In a second study (Exp. 2) to evaluate how maternal nutrition impacts offspring in an industry applied setting, 67 Angus cross cows received 0.75 kg/head/d of pellet supplying 12 g/d of rumen-protected methionine (**MET**, Smartamine[®]M), or a similar pellet without additional methionine (**CON**). Maternal diets for both studies were fed for the last eight weeks of gestation. After weaning, steer offspring (Exp. 1 n=55, Exp. 2 n=34) were managed as a group in the feedlot and fed common diets formulated to meet or exceed the requirements for growing and finishing beef steers (NASEM, 2016). Body weights were recorded every 14 days, blood samples were collected three days before slaughter, and carcasses were graded by a certified grader 24 hours after slaughter. Samples of neck muscle (*Sternomandibularis muscle*) were collected at slaughter for mRNA expression of genes associated with growth (*IGFs*, *INSR* and pyruvate kinase (*PKM*)) and the development of muscle (*MYOD* and *MYOG*) and fat (peroxisome proliferator activated receptor gamma (*PPARG*)) using real-time PCR (as described in Paradis et al., 2017; Hare et al., 2019). Data from the research-intensive and industry applied studies were analyzed separately using PROC GLIMMIX in SAS (University Edition, Version 9.4; SAS Institute Inc., Cary, NC) with maternal dietary treatments as the fixed effects, and steer and pen as the random effect.

Results and Discussion

Exp. 1: Steers from cows fed below or at MP requirements had higher (90%: 0.34 mmol/L, 100%: 0.39 mmol/L, 110%: 0.21 mmol/L; SEM = 0.041, *P* = 0.01) circulating non-esterified fatty acid (NEFA) concentrations, which may be indicative of increased fat mobilization (Webb

et al., 1969). Steers from cows fed at 90% MP also had higher (90%: 15.5 mm, 100%: 14.7 mm, 110%: 11.8 mm; SEM = 1.11, $P = 0.04$) carcass grade fat. These results support metabolic mismatch theory, in which undernutrition during gestation followed by diets that meet requirements postnatally increases fat development (Hales and Barker, 2001; Godfrey et al., 2007). Steers from cows fed over MP requirements had increased expression of *MYOG* (90%: 0.72, 100%: 0.94, 110%: 1.00; SEM = 0.083, $P = 0.02$), which is involved in muscle development (Paradis et al., 2017). Maternal MP level did not influence any other genes associated with growth ($P > 0.09$), fat ($P = 0.30$), or muscle ($P = 0.57$) development. Maternal methionine supplementation reduced the expression of *PKM* (RPM: 0.81, NoRPM: 1.16; SEM = 0.092, $P = 0.01$) in steer offspring muscle.

Exp. 2: Pyruvate kinase expression in offspring from methionine supplemented cows in the industry applied study had a higher (MET: 1.98, CON: 1.35; SEM = 0.221, $P = 0.05$) expression of *PKM* and was opposite of the effect in Exp. 1. This difference could be due to maternal management practices, as cows from the industry applied study had *ad libitum* access to their feed and dams from Exp. 1 feed intake was controlled. Steer offspring from MET cows also had consistently heavier body weight ($P \leq 0.02$); which may be reflective of increased *PKM* expression, which is associated with tissue growth (Hamelin et al., 2006) and muscle development (Lametsch et al., 2006). Additionally, steers from MET cows had increased (MET: 1.82, CON: 1.20; SEM = 0.190, $P = 0.02$) expression of *MYOD* which is also involved in muscle development (Paradis et al., 2017).

Conclusions

These studies show that maternal nutrition in late gestation influences beef steer offspring fat and muscle development. Feeding cows below MP requirements in late gestation, followed by diets that met requirements postnatally, influenced steer offspring fat development, which supports metabolic mismatch of the Barker hypothesis. Maternal nutrition in late gestation may prove to be a promising management opportunity to improve offspring development, carcass quality, and may lead to more AAA Canadian steaks.

Acknowledgements

We would like to thank the Elora Beef Research Centre, New Liskeard Agricultural Research Station, and University of Guelph Meat Lab staff, as well as all laboratory technicians for their assistance in this project. We would also like to thank the Natural Sciences and Engineering Research Council of Canada, Agriculture and Agri-Food Canada, and Beef Farmers of Ontario for their funding support.

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Contributions of Enzyme Technology to Poultry and Swine Nutrition

Contributions de la technologie des enzymes à la nutrition des monogastriques

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Abstract

There is a widespread acceptance and encouragement of use of exogenous enzymes in poultry and swine nutrition due to their benefits. Although the benefits are unequivocal for some enzymes, for others, the benefits are inconsistent. Some of these benefits include improvements in nutrient utilization and reductions in negative effects of feedstuff-resident antinutritional factors. Feed accounts for 70% of the cost of commercial production of poultry and swine, the addition of enzymes ensures that a higher proportion of nutrients in the feed are utilized. It also encourages the use of low-cost ingredients and protects the environment by reducing the amount of nutrients present in manure. Commonly used enzymes in monogastric nutrition include phytases, carbohydrases (xylanase, β -glucanase, and amylase), and proteases. These enzymes support the intestinal hydrolysis and utilization of minerals, carbohydrates, fiber, and proteins present in feed ingredients and to varying extent have been proven to increase the production of poultry and swine. Although there are inconsistencies with some of these enzymes particularly carbohydrases and proteases, there is ongoing concerted efforts aimed at improving the efficiency of these enzymes. Genetic modifications of microbes and enzyme immobilization are some techniques being used to improve the efficacy, thermostability, specific activity, and storage stability of these enzymes. More recently, multienzyme complexes have gained increased interests in improving enzyme efficacy by capitalizing on the additive effects of the enzymes, and in reducing production cost accrued through the utilization of individual enzymes products. In conclusion, enzyme technology has contributed immensely to modern poultry and swine production and will continue to play a role in ensuring food security and sustainable agriculture.

Key words: enzymes, nutrition, poultry, swine, techniques

Résumé

L'utilisation d'enzymes exogènes dans l'alimentation des volailles et des porcs est largement acceptée et encouragée en raison des avantages que cela procure. Bien que les avantages soient sans équivoque dans le cas de certaines enzymes, pour d'autres, ils sont inégaux. Au nombre de ces avantages, on compte des améliorations dans l'utilisation des nutriments et certaines réductions des effets négatifs des facteurs antinutritionnels présents dans les aliments pour animaux. L'alimentation représente 70 % des coûts de production des volailles et des porcs, et l'ajout

d'enzymes garantit qu'une proportion plus élevée des nutriments des aliments sont utilisés. Cette pratique favorise également l'utilisation d'ingrédients à faible coût et protège l'environnement en réduisant la quantité de nutriments présents dans les déjections. Les enzymes couramment utilisées dans la nutrition des monogastriques comprennent les phytases, les carbohydrases (xylanase, β -glucanase et amylase) et les protéases. Ces enzymes contribuent à l'hydrolyse et à l'utilisation intestinales des minéraux, des hydrates de carbone, des fibres et des protéines présents dans les ingrédients alimentaires et, à des degrés divers, il a été prouvé qu'elles augmentent la production des volailles et des porcs. Bien qu'il y ait des incohérences avec certaines de ces enzymes, en particulier les carbohydrases et les protéases, des efforts concertés sont actuellement déployés pour améliorer l'efficacité de ces produits. La modification génétique de micro-organismes et l'immobilisation des enzymes comptent parmi les techniques utilisées pour améliorer l'efficacité, la thermostabilité, l'activité spécifique et la stabilité de conservation de ces enzymes. Plus récemment, les complexes multi-enzymatiques ont suscité un intérêt accru pour l'amélioration de l'efficacité des enzymes car ils permettraient de profiter des effets additifs des enzymes et entraîneraient une diminution des coûts associés à l'utilisation de produits enzymatiques individuels. En conclusion, la technologie des enzymes a énormément contribué à la production moderne de volaille et de porc et continuera à jouer un rôle dans la sécurité alimentaire et l'agriculture durable.

Mots Clés: enzymes, nutrition, volaille, porcs, techniques

Introduction

The use of enzymes in modern non-ruminant production has become universal. This is due to the extensive benefits observed on the productivity of both poultry and swine with its use. Although most feed ingredients used in monogastric nutrition contain adequate nutrients, monogastric animals are not able to efficiently utilize all the nutrients present in these ingredients. This has been attributed to several factors including the presence of antinutritional factors (ANF) in several feed ingredients such as non-starch polysaccharides (NSP) in grains (wheat, barley, oats) and phytate in most oilseeds and cereals (Olukosi et al., 2007a). Similarly, pigs and poultry have inadequate endogenous enzymes required to hydrolyze certain components of feed ingredients including fiber, phytate-complexes and proteins thus, approximately 25% of feed passed through their intestinal tract remain undigested (Barletta et al., 2011). This has led to the loss of nutrients through manure resulting in environmental concerns such as eutrophication. Exogenous enzymes have been increasingly supplemented in feed to degrade naturally occurring ANF and increase the utilization of nutrients by animals. In addition, enzymes have been used to reduce the viscosity of digesta in the gut when cereals such as wheat or barley are fed to pigs and poultry (Le et al., 2013). Furthermore, due to the rising costs of corn and soybean, cheaper low-quality feed ingredients such as high-fiber feedstuffs and industrial by-products have seen increasing use in diets of pigs and poultry. This is partly due to the ability of enzymes to increase the bioavailability of nutrients in these ingredients to animals hereby raising their value as alternative feed materials. Lastly, the use of enzymes has proved effective in reducing the nutrient load of manure from pigs and poultry production thus reducing the negative impacts on the environment (Nahm, 2005).

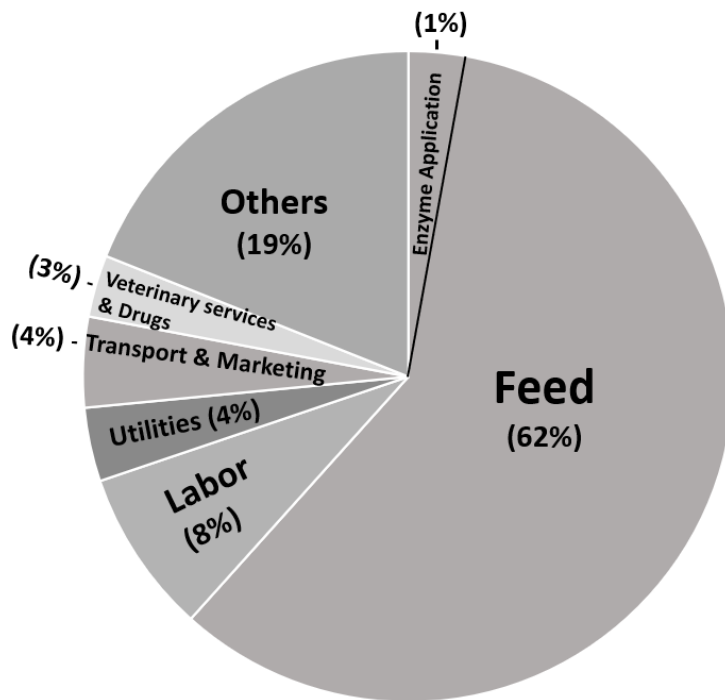


Figure 1. Contribution of enzyme application to the feed cost. Pie chart represents the total production cost per pig sold and is based on the production level of 18.6 finished pigs sold/sow/year. (Adapted from Dhuyvetter et al., 2014).

Feed comprises about 60-70% of the total cost of commercial production with exogenous enzymes accounting for approximately 1% of that cost (Figure 1). Commercially available feed enzymes for pig and poultry include several phytases, carbohydrases, and proteases targeted at the phytate, fiber, and protein components of feeds, respectively. Although these enzymes have recorded varying degrees of success in improving the utilization of nutrients in feed and increasing the productivity of poultry and swine, they are not yet at optimum efficiency. There are ongoing concerted efforts targeted at improving the production, stability, storage, and efficiency of these enzymes through innovations and technology (Menezes-Blackburn and Greiner, 2014). Therefore, the purpose of this paper will be to briefly review some of the commercially important enzymes in monogastric nutrition and examine the technologies being employed to improve their efficacy and utilization in poultry and swine production.

Phytase

Phytase (myo-inositol hexakiphosphate phosphohydrolase) is one of the most commercially successful exogenous enzymes in the market. This is due to its proven ability in breaking down phytate and its complexes and releasing phosphorus (P) and other nutrients for use by pigs and poultry (Selle and Ravindran, 2007). Phytase was first examined in the 1900s and was discovered as naturally occurring in plants materials such as rice and wheat bran. They were also found

endogenously in animals and could be extracted from microbes (Wodzinski and Ullah, 1996). Previously, microbial phytases were obtained from the fungus *Aspergillus sp.* However, current phytases in the market are obtained from the fermentation of bacteria species such as *Escherichia coli*, *Buttiauxella sp.*, and *Citrobacter braakii* (Menezes-Blackburn and Greiner, 2014). These phytases are usually classified as 3- or 6-phytase, depending on the position of the carbon from which the hydrolysis of the phosphate group begins (Zyla et al. 2004). In addition, these phytases operate in the gut using the histidine acid phosphatase catalytic mechanism (Greiner and Konietzny, 2011).

Phytate (myo-inositol hexakiphosphate) is the main storage form of P in most cereals, legumes, and oilseeds (Babatunde et al. 2019a). Due to the negative charge on the phytate inositol structure, strong complexes are formed with positively charged ions such as calcium (Ca), zinc, magnesium, and amino acids (AA) in the gut, making them unavailable for use by pigs and poultry (Cowieson et al. 2016). Phytase hydrolyzes phytate-P in a stepwise manner to its lower inositol phosphate esters, orthophosphates, and then further into myo-inositol (Figure 2) with the support of endogenous phosphatases (Cowieson et al. 2016). Phytases can be categorized as acidic, neutral, or alkaline depending on the optimum pH of activity (Greiner and Konietzny, 2011). However, most microbial phytases used in monogastric nutrition are acidic and active in the upper section of the gastrointestinal tract (GIT).

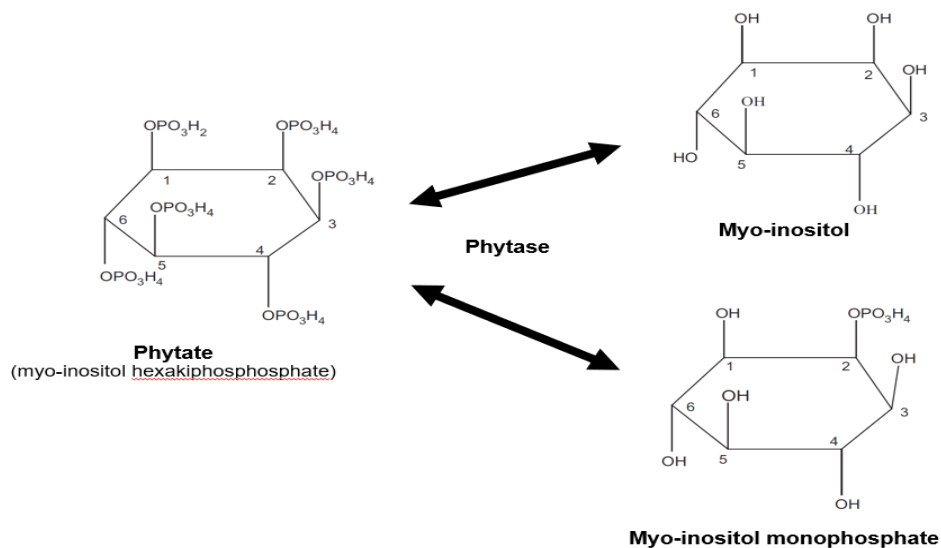


Figure 2. Schematic action of phytase enzyme on phytate substrate

Previous studies have reported an increase in body weight gain, feed intake and feed efficiency when phytase was supplemented in diets of broiler chickens (Shirley and Edwards, 2003; Babatunde et al., 2020), ducks (Adeola, 2018), laying hens (Taylor et al., 2018) and pigs (Olukosi et al., 2007b). Similarly, several studies have reported increase in the utilization of P, Ca, nitrogen, AA and energy when phytase was supplemented in the diets of poultry (Selle and Ravindran, 2007; Babatunde et al. 2019a,b) and pigs (Dungelhoeft et al., 1994; Adeola et al., 2006). Phytase has also been reported to improve the deposition of minerals on the bones of broiler chickens and pigs (Santos et al., 2014; Babatunde et al., 2020) and to reduce the P content of manure from pigs and

poultry (Nahm, 2005). However, the effectiveness of phytase supplementation in monogastric nutrition could be affected by several factors including the intrinsic properties of feed ingredients, the Ca-P relationship in the diet and gut, particle size of Ca sources in the diet, age of animals, length of feeding, and species of animal (Menezes-Blackburn and Greiner, 2014; Babatunde et al., 2019a).

Carbohydrase

The term carbohydrase is a collective name for a group of enzymes which include xylanase, cellulase, glucanase, α -amylase, β -mannanase, α -galactosidase, and pectinase. Fibrous feed stuff such as wheat, rye or barley meal are made up of overlapping layers of different structural carbohydrates called NSP which must be unfolded in order for digestive enzymes to gain access to the trapped nutrients (Petry and Patience, 2020). Moreover, considerable amount of energy yielding nutrients and minerals are located in the aleurone layer, typically made up of NSP that include beta-glucans, pentosans, oligosaccharides, cellulose and lignin. However, they are protected from hydrolysis in the digestive tract since pigs and poultry do not secrete appropriate enzymes capable of degrading them hereby resulting in inefficient use of these feed stuff (Bach and Knudsen, 1997). High dietary NSP increases digesta viscosity, which reduces enzyme accessibility to substrates and slows down the rate of digestion.

Supplementing diets with carbohydrases reduces digesta viscosity via a decrease in feed polymerization and release of carbohydrate oligomers for improved nutrient utilization (Kalmendal and Tauson, 2012; Guo et al., 2014). The pig gut, compared to poultry, is better equipped to digest, and utilize a portion of the complex carbohydrates due to a relatively larger digestive tract, longer digesta residence time, and greater bacteria fermentation in the hind gut (Knudsen et al., 2012). However, changing composition of swine diet to include more fibrous ingredients and associated complexities of the target substrate creates an avenue for improvements in nutrient utilization by use of carbohydrase supplementation. Certain enzymes, like xylanase, mannanase and glucanase may be exogenously supplemented as feed additives to specifically target NSP hydrolysis. This unfolds the cell wall and grants other digestive enzymes like amylase access to nutritional constituents, such as protein, starch, lipids, and other minerals, trapped within the cell-wall matrix (Le et al., 2013). Some specific carbohydrases including their mode of action are discussed below.

Xylanase

Arabinoxylans are major constituents of the cell wall of plants like barley, oats, wheat, rye, and their by-products. This complex polysaccharide is mainly composed of two pentoses: arabinose and xylose and can affect nutrient utilization in pigs and poultry by forming viscous gels in the animal's gut. Although majority of feed ingredients for livestock have high starch content, it is stored in granules within a matrix of storage proteins and enclosed by the thin cell walls of the starchy endosperm that mainly consist of arabinoxylans (Evers and Millar, 2002). This is referred to as the arabinoxylan cage, or the caging effect (Figure 3). There are several reports on improved feed digestibility in broiler chickens and pigs fed xylanase supplemented diets (Kalmendal and Tauson, 2012; Guo et al., 2014; Petry and Patience, 2020). It is broadly accepted that xylanases achieve this by two main functions: One is to decrease viscosity in the gut triggered by the water-soluble NSP and thereby facilitate the digestive and absorptive processes in the GIT. Another is to

degrade the β -,4-glycosidic bonds of the xylan backbone of arabinoxylan to produce branched or non-substituted xylo-oligosaccharides that could potentially be used by the animal (Le et al., 2013). The disruption of the structural integrity of the cell walls opens up the “cage” for digestive enzyme access (Figure 3).

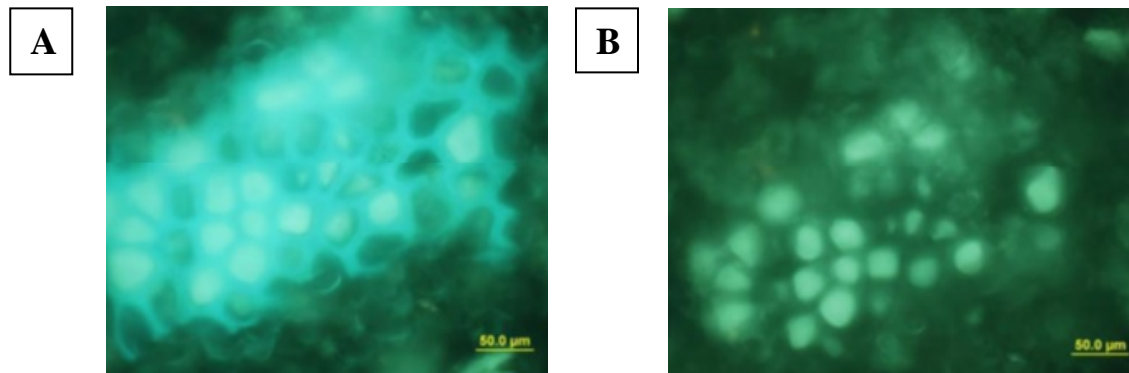


Figure 3. Microscopic visualization of the degradation of aleurone arabinoxylan cages present in milled wheat after incubation with Ronozyme WX xylanase (1 g of enzyme/kg of diet) at 30°C. Images depict a close-up of a cell wall structure containing ferulic acid, covalently bound to cell walls, and which fluoresces with an intense blue-green fluorescence. Panel A and B shows cell walls before and after addition of the xylanase, leading to the breakdown and disappearance of the cell wall architecture. (Adapted from Le et al., 2013)

Amylase

Starch is the major energy storage in plants, and is a polymeric carbohydrate consisting of numerous glucose units joined by glycosidic bonds. Generally, swine and poultry appear to secrete sufficient pancreatic amylase to cater for dietary starch intake. However, factors not directly related to starch itself, such as specific intestinal site may also affect its digestibility (Aderibigbe et al., 2020a). Similarly, the dynamics of starch digestion relative to intestinal efficiency and age may have considerable nutritional consequences (Aderibigbe et al., 2020a,b). Starch degradability is also affected by the proportion of amylose in feedstuff, its variability in corn and other cereal grains can significantly influence the ME content of feedstuffs to livestock (Tester et al., 2004). Some can escape enzymatic digestion in the GIT (also called resistant starch) and may only be fermented in the hindgut by microbes (Tiwari et al., 2019). However, fermentation in the distal gut is a much less efficient usage than the breakdown in the proximal regions of the GIT. Particularly in young chickens, exogenous amylases may augment the function of endogenous amylase which is limited by the maturation of the GIT (Croom et al., 1999), but this is not always true. The additional benefits from exogenous amylases in poultry and swine nutrition have been inconsistent largely due to the relatively high innate starch digestion capacity of the animal. Therefore, amylase supplementation as part of a carbohydrase mix containing xylanases or glucanases have been reported to improve energy utilization and the performance of non-ruminant animals (Olukosi and Adeola, 2008; Schramm et al., 2021).

Protease

Leguminous seeds such as peas and soybeans, contain large amounts of storage proteins essential for optimal animal growth and development. However, they also contain high concentrations of ANF (Table 1). Dietary protein is degraded by proteases produced in the GIT; the most important are pepsin, trypsin, and chymotrypsin. Anti-nutritional factors such as trypsin inhibitor (TI) contained in these seeds, inhibit protein digestion by blocking the action of endogenous trypsin critical to the activation of other digestive enzymes in the gut (Erdaw et al., 2017). This negatively affects nutrient utilization and animal performance. Furthermore, increased inefficiency of dietary protein utilization exacerbates the environmental impact of high nitrogen emissions (Leinonen and Williams, 2015). Although the inhibitors are heat labile and are commonly deactivated by heat treatment, excessive processing can negatively influence the nutritional quality of the feed (Newkirk, 2010).

Table 1. The trypsin inhibitor activity (TIA) of some legume seeds. (Adapted from Avilés-Gaxiola et al., 2018).

Scientific name	Common name	TIA (U/mg)
<i>Glycine max</i>	Soybean	94.1
<i>Phaseolus vulgaris</i>	Kidney bean	3.10
<i>Lens culinaris</i>	Lentil	7.40
<i>Vigna unguiculata</i>	Cowpea	7.52
<i>Arachis hipogea</i>	Peanut	5.60
<i>Cicer arietinum</i>	Chickpea	12.1

Exogenous proteases can therefore be an option to improve performance of the animal while reducing dietary protein levels. This is achieved through enhanced protein solubilization and hydrolysis of dietary proteins (Zuo et al., 2015; Aderibigbe et al., 2020c). Microbial protease could also destroy or inactivate the TI in soybean (Huo et al., 1993). Because TI are competitive substrate analogs of trypsin, hydrolysis of TI by exogenous proteases may allow for increased trypsin activity in the GIT. This potentially improves the utilization of dietary protein and AA by pigs and poultry, and consequently improves animal performance (Zuo et al., 2015; Aderibigbe et al., 2020c). There are also observed responses that suggest extra-proteinous effect of protease, extending beyond a magnitude that is commensurate with increases in the digestibility of protein alone (Olukosi et al., 2015; Cowieson et al., 2017). This may be associated with the disruption of protein-starch matrix in the feed following proteolysis. However, the efficacy of exogenous proteases in poultry and swine nutrition is largely inconsistent and has been attributed to the different inherent characteristics of commercial proteases which may elicit divergent responses in the animal. This may also be related to compatibility with endogenous proteases, changing chemical gut environment during the digestive process, or perhaps modification of the seed protein during processing (Cowieson and Roos, 2016). Nevertheless, considerable opportunity exists to

develop novel proteases which are more functional at low pH or which specifically target proteinaceous antinutrients such as lectins, TI or other antigenic proteins.

Table 2. Responses of non-ruminant animals to single or multi-enzyme supplementation in diets.

S/N	Enzyme	Specie	Response	Reference
1	Phytase	Broiler chicken, Pig, Laying Hen, Duck	Improved growth performance, nutrient digestibility, bone mineralization, egg quality	Adeola, 2006; Santos et al., 2014; Adeola, 2018; Taylor et al., 2018; Babatunde et al., 2019a,b; 2020.
2	Xylanase	Broiler chicken, Pig	Improved nutrient digestibility, reduced digesta viscosity.	Guo et al., 2014; Petry and Patience, 2020
3	Amylase	Broiler chicken	Improved growth performance, nutrient digestibility	Aderibigbe et al., 2020a,b; Schramm et al., 2021.
4	Protease	Broiler chicken, Pig	Improved growth performance, amino acid digestibility	Zuo et al., 2015; Cowieson et al., 2017; Aderibigbe et al., 2020c
5	Xylanase + Protease	Broiler chicken	Improved growth performance, nutrient digestibility	Kalmandal and Tauson, 2012
6	Xylanase + Phytase	Broiler chicken, Laying hen, Pig	Improved growth performance, nutrient digestibility, egg quality, bone mineralization	Wu et al., 2004; Olukosi et al., 2007a; 2008; Taylor et al., 2018
7	Xylanase + Protease + Phytase	Broiler chicken	Improved growth performance, net energy, starch, and amino acid digestibility.	Cowieson et al., 2019
8	Xylanase + Amylase + Protease	Broiler chicken	Improved growth performance, nutrient digestibility.	Olukosi et al., 2007a; 2015
9	Xylanase + Amylase + Protease + Phytase	Broiler chicken, Pig	Improved growth performance, nutrient digestibility.	Olukosi et al., 2007b; 2015

Single versus Multi-enzyme matrix

Conventionally, enzymes are administered as single additives in animal diets. In recent times, additional benefits have been reported for enzymes added to diets as part of a multi-enzyme cocktail or combined as stand-alone additive in the complete diet (Table 2). Although the potency of single enzymes is not in doubt, the use of multiple enzyme activities simultaneously targets multiple substrates i.e., attack different antinutritive compounds or break up nutrient complexes in feedstuffs to obtain the maximum benefit from the enzymes. Therefore, thorough knowledge of how enzymes work together to hydrolyze their respective substrate is essential to maximizing the efficacy of enzyme combinations. For instance, xylanase increases the permeability of the aleurone layer of wheat, which is also the site of phytic acid storage (Parkkonen et al., 1997). This suggests that supplementing animal diet with a combination of xylanase and phytase may be mutually beneficial to the animal. In contrast, there are reports that suggest that proteases contained in enzyme mixtures may degrade or limit the activity of other constituent enzymes (Saleh et al., 2004). Other works reported no additional benefit of using enzyme combinations compared with using enzymes individually (Wu et al., 2004; Olukosi et al., 2007a). This may be partly due to the complexity of the potential substrates, which also varies by cereal and diet formulation. Moreover, majority of the so-called 'single-component' enzyme products have ancillary side activities and thus interpretation of results using single enzymes should be treated with caution. Although it is possible that a multitude of enzyme activities is unnecessary or even detrimental, nevertheless there is substantial experimental data showing multi-enzymes to be effective in pigs and poultry (Olukosi et al., 2007a, b; Kalmandal and Tauson, 2012; Taylor et al., 2018; Cowieson et al., 2019).

Advances in Enzymes Technology

The use of enzymes in monogastric nutrition is not without challenges. One of which is the enhancement of enzyme properties for specific feed applications. Enzymatic properties such as thermostability, specific activity, storage stability, and resistance to the harsh environment of the GIT, are areas of interest in ongoing efforts to improve enzyme technology (Menezes-Blackburn and Greiner, 2014). New enzyme products with improved properties are being developed through the screening of extremophiles or other thermophilic organisms capable of living in hostile environments. Extremophiles have the capacity to thrive in areas with extreme temperatures, salinity, and pressure thus, isolation and production of these microbes may generate enzymes with similar properties (Iyer and Ananthanarayan, 2008). Another approach is the modification of existing microbes through modern biotechnological applications such as large-scale fermentation and recombinant DNA technology (Cherry and Fidantsef, 2003). This could result in the production of recombinant enzymes at maximal purity and with economic efficiency. Protein engineering, directed evolution, mutation, and screening of genes from current microbes may also improve specific enzyme properties such as thermostability and substrate specificity (Cherry and Fidantsef, 2003). A good example has been the construction of a 'consensus phytase'. This is a combination and alignment of 13 phytic enzymes which has been reported to be more efficient and thermostable than the parent enzyme and has been regarded as a success in the phytase industry (Lehmann et al., 2002). Chemical modification of enzymes involving the alteration of AA side chain structures, could improve stability. However, the use of chemical cross-linking may not always result in the improved stability of the final enzyme product (Davis, 2003).

Other common and preferred approaches include the use of immobilization techniques in improving the thermo- and storage stability of enzymes (Iyer and Ananthanarayan, 2008). These techniques include adsorption, membrane confinement, entrapment, and covalent binding of which multipoint covalent attachment is the most effective in improving the thermostability of enzymes (Guisan et al., 1993). Coating of enzymes has been reported to improve stability and reduce the possibility of dustiness (Menezes-Blackburn and Greiner, 2014). In addition, the use of additives in enzyme preparations has proven reliable overtime in improving the storage stability of enzymes. Some effective additives include ligands, salts, polyols, sugars, and synthetic polymers (Iyer and Ananthanarayan, 2008). Similarly, feed processing conditions and raw material pretreatments could be means of improving efficacy of enzymes. High temperatures during pelleting of feed have been reported to negatively impact the activity of enzymes (Inborr and Bedford, 1994). However, post-pelleting applications of enzymes may ameliorate some of these effects. Developing pretreatment procedures for the hydrolysis of hemicellulose fraction cereals, high-fiber ingredients, and high-phytate feed materials may prove to be of economic importance as they will increase the efficacy of enzymes in nutrient extraction (Marquardt and Bedford, 2001). There have been efforts to determine the site of peak enzyme activity in the gut of pigs and poultry particularly with phytases (Rodehutsord and Rosenfelder, 2016). This information has helped produce enzymes with properties targeted at being most efficient in those sections of the GIT. Lastly, transgenic animals with the capacity to secrete enzymes in their saliva, effectively utilize nutrients in feed, and produce low-nutrient manure are being produced and investigated (Zhang et al., 2018). However, this technology faces challenges from consumer perspectives, ethical groups, and various legalities and will require time to become fully accepted.

Future Considerations

Research targeted at improving enzyme assays remains essential as there is no universal standard procedure for analyzing the quantity or quality of the different commercial enzyme products. Enzyme products generally have differing characteristics such as optimal temperature and pH, storage stability, and substrate specificity. Thus, determining the activity level in feeds and the comparison of various commercial products is challenging. Moreover, developing a standardized single enzyme assay will allow producers and purchasers of enzymes compare products based on relative activity, allow assessment of enzymes that survive feed processing procedures, and evaluate the survivability of enzymes in the GIT (Marquardt and Bedford, 2001). Further investigations into the relationship and interactions between the gut microbiome and existing enzymes may go a long way in explaining some of the inconsistencies observed with enzyme efficacies particularly with carbohydrases and proteases. Additionally, it may prove invaluable to further evaluate the exact structural conformation of the substrates to be degraded as this information will be important in developing the next generation of enzymes.

Conclusions

Enzyme technology has played a fundamental role in the increased productivity of monogastric animal production observed today. The ability of enzymes to improve the utilization of nutrients from feed ingredients, increase the production of pigs and poultry, while reducing their environmental impact has proven invaluable. Currently, the feed enzyme market is estimated to be

worth \$1.3 billion and it is projected to reach \$1.9 billion by 2025. A large share of this market is dominated by the use of phytases in the diets of pigs and poultry followed closely by carbohydrases and proteases. To keep up with the projected growth in the world population, and the consequent increase in commercial non-ruminant production, efforts should be targeted at overcoming challenges with the inconsistencies in animal responses to exogenous enzymes. In addition, the development of economical and effective next-generation enzymes should be encouraged for supporting sustainable animal agriculture.

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Abstract

Automation can be defined as technology that allows for a process to be performed automatically, with minimal human input. In the dairy industry the use of automated technology in feeding of dairy cattle is expected to grow, being necessitated to improve the accuracy and precision of diets fed in effort to improve health, production, and efficiency, as well as to minimize the requirements for human labor input. Improved consistency, or precision, of diets provided to lactating dairy cows may have significant impacts on herd-level performance. Measures to reduce ration variability, such as implementation of automation in diet preparation, may lead to improvements in herd performance and profitability. Automation may also be important for delivery of diets, as well as in maintaining continual access to feed. These are important for maintaining consistency in diet consumed throughout the day. In addition to herd-level feeding precision achieved through automation, there is also opportunity to use automated technology to achieve that nutrition precision at a cow and calf level. The rapid adoption of technologies that allow for individualized feeding, including automated milking and calf feeding technologies, has also increased our ability to feed cattle according to their individual requirements. While there are still many challenges associated with the successful implementation of such precision feeding strategies, on-going research would suggest that these opportunities will continue to grow and be refined.

Résumé

L'automatisation peut être définie comme une technologie qui permet d'exécuter un processus automatiquement, avec un minimum d'intervention humaine. Dans l'industrie laitière, l'utilisation des technologies d'automatisation pour l'alimentation des animaux devrait se répandre, car celles-ci seront nécessaires pour accroître la précision des rations afin d'améliorer la santé, la production et l'efficacité ainsi que pour diminuer les besoins en main-d'œuvre. L'augmentation de la fiabilité ou de la précision des rations servies aux vaches laitières en lactation pourrait avoir d'importantes répercussions sur les performances du troupeau. Les interventions visant à réduire la variabilité de l'alimentation, telles que l'automatisation de la préparation des rations, peuvent améliorer les performances et la rentabilité du troupeau. L'automatisation peut aussi jouer un rôle significatif dans la distribution des rations ainsi que pour assurer l'accès continu aux aliments. Ces éléments sont importants pour maintenir l'uniformité de la ration consommée durant la journée. Outre la précision de l'alimentation de l'ensemble du troupeau obtenue grâce à l'automatisation, il est également possible d'utiliser des technologies d'automatisation pour obtenir le même degré de

précision à l'échelle de la vache et du veau. L'adoption rapide de technologies qui permettent une alimentation individualisée, notamment les technologies de traite et d'alimentation des veaux automatisées, a également accru notre capacité à nourrir le bétail en fonction de ses besoins individuels. Bien qu'il reste de nombreux défis à relever pour mettre en œuvre avec succès de telles stratégies d'alimentation de précision, les recherches en cours semblent indiquer que les possibilités continueront à se développer et à s'améliorer.

Introduction

Automation can be defined as technology that allows for a process to be performed automatically, with minimal human input. The adoption and use of automation in the dairy industry has grown dramatically in the past decade. Much of the automation adopted has been associated with milking and other aspects of barn management. There is also growing interest and adoption of automation in feeding of dairy cattle. Specifically, the use of automation in feeding of lactating dairy cows is growing, being necessitated to improve the accuracy and precision of diets fed to dairy cows in effort to improve health, production, and efficiency, as well as to minimize the requirements for human labor input. Human labor for dairy operations may not only be increasingly hard to come by and expensive, but may also be more prone to error and inconsistency in comparison to automated technologies. Further, the rapid adoption of technologies that allow for individualized feeding, including automated milking and calf feeding technologies, has also increased our ability to feed dairy cattle according to their individual requirements, thereby facilitating precision nutrition. This paper will review the various needs for automation in feeding dairy cattle to achieve precision, outlining both opportunities and challenges.

Automation in Dietary Preparation

Improved consistency, or precision, of diets provided to dairy cows may have significant impacts on herd-level performance. Measures to reduce ration variability, such as implementation of automation in diet preparation, may lead to improvements in herd performance and profitability. The need for automation is highlighted by the fact that, despite best efforts, the delivered ration on many dairy farms does not accurately match that which was formulated for the cows. In previous research we observed that as the variability between the ration offered to the cows and the original formulated ration becomes greater, so does the chance that cows will not perform to expectation (Sova et al., 2014). While most of us have always suspected that cows do not always receive the ration exactly as it is formulated for them, this research is some of the first to support this idea and identify the potential consequences of such deviations. For our study we sampled the mixed and delivered total mixed ration (TMR) for 22 free-stall, parlor-milked herds for 7 consecutive days in the winter and summer months. The nutrient analysis of these feed samples was then compared to that formulated on paper for those farms. Across farms, the average TMR fed did not accurately represent that formulated by the nutritionist. The average TMR delivered exceeded TMR formulation for net energy of lactation (NE_L), non-fibre carbohydrate (NFC), acid detergent fibre (ADF), calcium, phosphorus, magnesium and potassium, and underfed crude protein (CP), neutral detergent fibre (NDF) and sodium. Theoretically, some deviation might not be hugely problematic because a safety margin is generally included in formulation to account for uncertainty in

ingredient composition. Across farms, however, there was a huge range in this variation, with some farms consistently experiencing a 5–10% discrepancy (both positive and negative) between the fed and formulated ration for nearly all nutrients. This lack of dietary accuracy can be costly, either in excessive provision of expensive ingredients, or not allowing cows to meet their production expectations due to nutrient shortfall.

In that study we also investigated the day-to-day consistency in physical and chemical composition of TMR and associations of this variability with measures of productivity. Greatest day-to-day variability was observed for refusal rate, particle size distribution, and trace mineral content. Delivery of a more consistent ration was associated with improved production. For example, greater herd-average dry matter intake (DMI; Figure 1), milk yield (Figure 2), and efficiency of milk production were all associated with less daily variability in energy content of the ration (Sova et al., 2014).

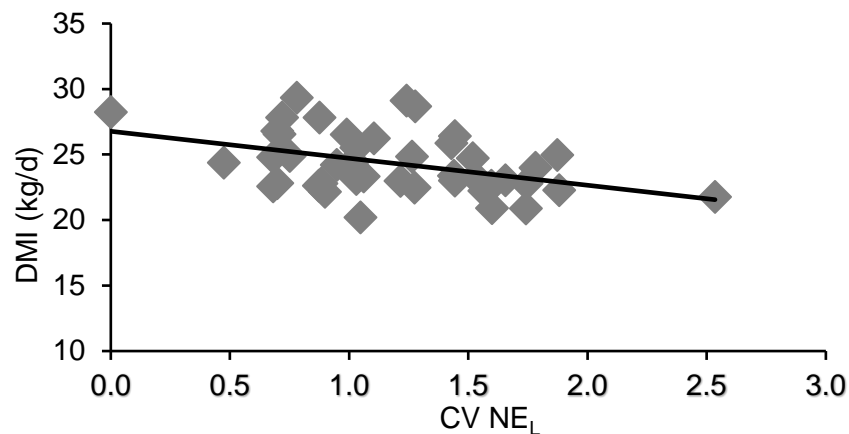


Figure 1. Association between fed ration coefficient of variation (CV) in NE_L and average DMI. Coefficient of variation was calculated as the standard deviation of NE_L over 7 d divided by the average NE_L over 7 d. Figure adapted from Sova et al. (2014).

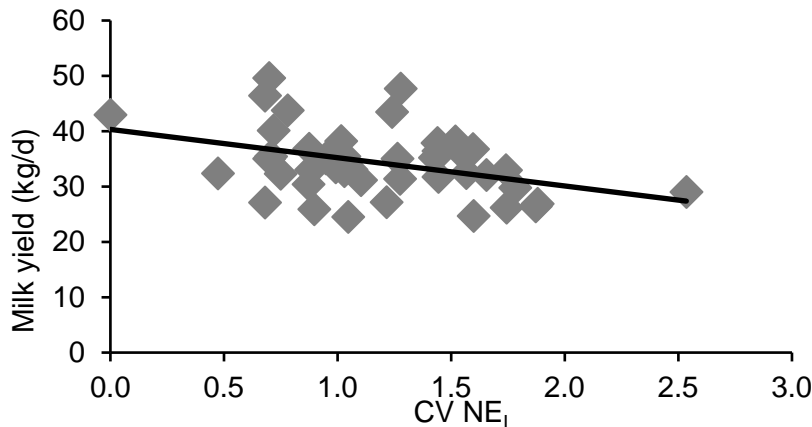


Figure 2. Association between fed ration coefficient of variation (CV) in NE_L and test-day milk. Coefficient of variation was calculated as the standard deviation of NE_L over 7 d divided by the average NE_L over 7 d. Figure adapted from Sova et al. (2014).

Lower day-to-day variation in the percentage of long forage particles in the offered TMR was also associated with greater milk yield and efficiency of milk production (Sova et al, 2014). On average, day-to-day variability was greater for physical characteristics (i.e., particle size distribution) of the ration compared with the ration's nutritional composition. This suggests that this day-to-day variation may have been caused by variability in feed component nutrient and dry matter (DM) composition, but probably even more so by mixing errors associated with operators (timing, sequencing) or equipment. Regardless, these findings suggest that increased surveillance of the TMR composition, in addition to individual feed ingredients (e.g., regular, frequent forage DM determination, regular nutrient testing of feeds), may be helpful as a regular component of feeding management to ensure delivery of TMR with the intended nutrient composition to maintain production and feed intake. Further, these findings reinforce the need for standard feeding protocols and training to achieve those protocols.

It is noteworthy that all of these practices to ensure dietary accuracy and precision may be enhanced through use of automated technologies. For example, real time sensors (e.g. NIRS) can be used on feed preparation equipment to determine dry matter and nutrient content of feed ingredients being incorporated into diets. TMR management programs may be used for more accurate weighing of ingredients, easier adjustment and calculation of pen delivery amounts, and monitoring of mix consistency (both timing and composition). Finally, automated feeding systems are available, which may be setup to fully automate the collection of dietary components and mixing of those together into a TMR.

Automation in Diet Delivery and Feed Access

Automation may also be important for delivery of diets, as well as in maintaining consistent and continual access to feed, which are key components in maintaining precision of nutrient consumption. The diurnal feeding patterns of dairy cows fed a TMR are primarily influenced by the time of feed delivery, feed push-up, and milking (DeVries et al., 2003). Of these, the delivery of fresh TMR has the single largest impact on stimulating feeding activity at the bunk (DeVries and von Keyserlingk, 2005; King et al., 2016a). As a result, greater frequency of feed delivery can greatly influence feeding behavior patterns, promoting more consistency in feed activity across the day (DeVries et al., 2005). In some studies, greater frequency of TMR delivery has also been associated with greater DMI (Sova et al., 2013; Hart et al., 2014). Further, delivering a TMR 2x/d or more often has also been demonstrated to reduce the amount of feed sorting compared with feeding 1x/d (DeVries et al., 2005; Endres and Espejo, 2010; Sova et al., 2013), which would further contribute to more consistent nutrient intakes over the course of the day. Such desirable feeding patterns are conducive to more consistent rumen pH, which likely contributes to improved milk fat (Rottman et al., 2014). In support of that, Woolpert et al. (2017) reported that dairy herds with high de novo fatty acid concentration in bulk tank milk, compared with those with low de novo fatty acid concentration, tended to be 5x more likely to be fed 2x versus 1x per day, confirming the positive impacts of feeding >1x/d on maintaining a consistent rumen environment.

Implementation of greater TMR delivery frequency on dairies is often constrained by time and cost associated with TMR preparation and its delivery. Thus, implementation of feeding

automation, not only for diet preparation, but also for frequent delivery to cows across the day may have significant benefits in terms of achieving greater precision. There is, however, a paucity in research on the effectiveness of the implementation of these automated TMR mixing and delivery systems.

TMR push-up is also critical to ensure that feed is accessible when cows want to eat. Feed push up needs to occur frequently enough such that any time a cow decides to go to the feed bunk, there is feed available to her. Feed push-up also helps minimize variation in feed consumed because it mixes up the feed that is no longer in reach with that which is currently available in the bunk. Thus, frequent pushing up of TMR in the bunk is necessary, particularly in the first few hours after feed delivery, when the bulk of the feeding activity has occurred. We have demonstrated that greater lying duration is associated with greater frequency of feed push-ups (Deming et al., 2013; King et al., 2016b), suggesting that frequent push-up minimizes the time cows need to spend waiting for feed access and cows can devote more time to lying down. Feed push-up will also ensure that DMI is not limited and thus production is optimized. Evidence for this was shown in a cross-sectional study of 47 herds, all with similar genetics and feeding the exact same TMR (Bach et al., 2008). In that study it was reported that those herds where feed was not pushed up (5 out of 47 herds) produced 3.9 kg/d/cow less milk (-13% difference) than herds where feed was pushed up. Interestingly, in a more recent observational study of robot herds, Siewert et al. (2018) reported that farms with automatic feed push-up produced 352 kg more milk/robotic unit and 4.9 kg more milk/cow per day than farms that manually pushed up feed. This effect may not be directly attributable to the use of an automated feed pusher, but rather that those farms using such automated equipment had more consistent feed push-up, and thus continuous feed access, than those pushing up feed manually. In support of this, in a recent observational study of 197 robot herds in Canada, we demonstrated that each additional 5 feed push-ups per day was an associated 0.35 kg/d greater milk yield at a herd level (Matson et al., 2021). Thus, in situations where manual feed push-up is done consistently and frequently, the same results should be achievable. Unfortunately, in reality, manual feed push-up, performed by farm staff, is more prone to inconsistency, in time and frequency; thus, this again provides supports for the use of automation.

Automation in Individualized Feeding

In addition to herd-level feeding precision achieved through automation, there is also opportunity to use automation to achieve that precision at an individual animal level. The rapid adoption of technologies that allow for individualized feeding, including automated milking and calf feeding systems, has also increased our potential ability to feed cattle according to their individual requirements.

Given the ability to supplement the feed consumption of dairy cows within automated milking systems (AMS), there is potential for applying some type of precision feeding approach in AMS (Bach and Cabrera, 2017). While there is potential, there are many challenges with such an approach. In AMS, the herd is fed a common diet (partial mixed ration – PMR) at the feed bunk. As this PMR diet is static, the ‘precision’ aspect, to meet individual cow nutrient needs, would need to be accomplished with the feed provided at the AMS. In theory, if the individual nutrient requirements were known (based on expected milk production, and other known factors including

age, body weight, stage of lactation, pregnancy status) then the amount of feed provided in the AMS could be adjusted to their individual need. The challenge with that is then also be able to accurately predict the nutrient consumption from the PMR, as that is not measurable on an individual basis in commercial settings. Therein lies some difficulty, it has been demonstrated that the level of PMR consumption is affected by the level of concentrate provided at the AMS, and it is not necessarily an even substitution ratio (Hare et al., 2018; Menajovsky et al., 2018; Paddick et al., 2019; Schwanke et al., 2019). In fact, across studies, the substitution rate (amount of decrease in PMR intake for every 1 kg increase in AMS pellet intake) has ranged from 0.63 to 1.58 kg (Hare et al., 2018; Schwanke et al., 2019). So, it may be difficult to predict total DMI, and thus total nutrient intake, when varying the amount of feed provided at the AMS, making precision feeding more difficult. Further, in studies where we have increased the quantity of AMS pellet offered in the AMS, the day-to-day variability in the consumption of the AMS pellet also increased (Hare et al., 2018; Menajovsky et al., 2018; Paddick et al., 2019; Schwanke et al., 2019). This variation then makes the concept of precision more difficult to attain. A further challenge with feeding in AMS is that just because cows are provided feed at the AMS, does not guarantee they will consume it (Bach and Cabrera, 2017). Any unconsumed feed left in the AMS results in another cow potentially consuming more than what they are programmed for; this reduces the ability to precision feed these animals.

While most AMS are only equipped with a single bin for delivering concentrate to cows (Bach and Cabrera, 2017), there is opportunity within many systems to provide multiple feeds. It is possible that greater precision in feeding could be achieved in such scenarios, as the amount and balance of different types of supplement feeds could be used to match individual cow nutrient requirements. To date, however, there is limited research on this type of approach. In a recent study, we demonstrated that we could improve energy balance and minimize body condition loss in early lactation by supplementing cows milked in AMS with a molasses-based liquid feed supplement in addition to their regular AMS concentrate (Moore et al., 2020).

There may be opportunities to apply such precision feeding principles in other types of milking systems. One such example is that described by Bach (2014), a 'dynamic concentrate parlor feeder' which involves the preparation and delivery (in real time) of many different feeds (in both quantity and composition) within a rotary milking parlor. The system calculates the individual nutritional requirements of each cow entering the parlor based on her assigned feed intake (average of the pen where she is), composition of the TMR fed, DIM, parity, BW, BW change, pregnancy status, milk yield, and milk component yields. Based on those needs, the system creates a least-cost formula using up to 6 feeds that are mixed and delivered to the cow in less than 14 seconds. Bach (2014) suggested that such a system would allow for the feeding of a more cost-effective TMR with a low nutrient density, without compromising, and even potentially improving, income over feed cost by delivering nutrients to only those cows in need of them.

One area where there has been more success in application of individualized feeding strategies is with the use of automated calf feeders. Automated calf feeders provide the ability to feed calves individualized diets that may be calf-specific based on age, weight, or any other parameter deemed appropriate. This may include altering the speed at which milk allowance is increased in early life, as well as decreased at the time of weaning. To date, however, much application of these feeding strategies, while applied at the calf level, is still done similarly across all animals

within a farm. There is research to suggest that much gain can be made by tailoring feeding programs for individual calves based on their individual needs. For example, de Passille and Rushen (2016) demonstrated that individual calves differ greatly in when they begin to consume solid feed and how quickly they increase the intake in response to a decrease in milk allowance. Those researchers demonstrated that automated milk feeders could be used to wean calves at variable ages, depending on their ability and willingness to eat solid feed.

Conclusions

Automated technologies have been developed and increasingly adopted within the dairy industry to not only reduce human labour requirements, but also to increase the accuracy and precision of application of various management tasks. Various forms of feeding technologies are currently available to increase our precision in feeding strategies of dairy cattle. At a herd-level, this includes automated feed preparation and delivery. While, at the animal level, this includes individualized feeding opportunities, to date primarily through automated milking in lactating cows and automated milk feeders in calves. While there are still many challenges associated with the successful implementation of such precision feeding strategies, on-going research would suggest that these opportunities will continue to grow, allowing for greater nutrient capture, greater efficiency, less nutrient waste, and greater health and production.

Acknowledgements

Much of the research presented in this paper was funded by the Natural Sciences and Engineering Research Council of Canada, Dairy Farmers of Canada, Agriculture and Agri-Food Canada, the Canadian Dairy Commission, Dairy Farmers of Ontario, the Ontario Ministry of Agriculture, Food, and Rural Affairs, Eastgen, the Canadian Foundation for Innovation, the Ontario Research Fund, and the University of Guelph.

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The Role of Mechanistic Models in Precision Dairy Nutrition and On-Farm Decision Support

Le rôle des modèles mécanistes en nutrition laitière de précision et comme outils d'aide aux décisions à la ferme

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Abstract

Models have a strong history of application in dairy production, where their objectives have revolved around optimally feeding and growing livestock. These predominantly nutritional (and typically quasi-mechanistic) models have evolved to mathematically express our cumulative biological knowledge, developed in order to understand and manipulate nutrient dynamics in the animal. In the field, these models serve as ‘decision support tools’ and are often linked to optimization algorithms to determine optimal ration composition. Academically, mechanistic models (MM) also serve to extract meaningful information from data and increase our biological understanding of complex systems. Current limitations of this modelling approach, in terms of application on-farm, revolve around their manual nature and lengthy input requirements. Meanwhile, the emergence of big data and associated machine learning (ML) approaches are taking the world (and some agriculture sectors) by storm, propelled by their quick development time and precision on-farm. No doubt they will have a major role to play in the future of on-farm decision support systems for dairy production. However, their slow adoption rate in animal production thus far may be due to the current degree of digitalization, the utility offered, return-on-investment (ROI), and/or the challenge of maintaining sensitive technology in corrosive, dusty and dirty environments. More than likely, the future of on-farm decision support will be a hybridization of these two approaches (MM and ML), utilizing the respective strengths and augmenting weaknesses of each approach. This talk will examine the use of MMs on-farm and in precision dairy, and contemplate future directions of precision dairy.

Résumé

L'application de modèles en production laitière remonte à loin, et leurs objectifs portent généralement sur l'optimisation de l'alimentation et de la croissance du bétail. Ces modèles essentiellement nutritionnels (et généralement quasi-mécanistes) ont évolué de manière à exprimer mathématiquement la somme des connaissances biologiques développées pour comprendre et manipuler la dynamique des nutriments chez l'animal. Sur le terrain, ces modèles servent d'« outils d'aide à la décision » et sont souvent liés à des algorithmes d'optimisation permettant de déterminer la composition optimale des rations. Sur le plan académique, les modèles mécanistes (MM) servent

également à extraire de l'information utile des données et à accroître notre compréhension biologique des systèmes complexes. Les limites actuelles de cette approche, en termes d'application à la ferme, viennent de la nature manuelle des modèles et du temps qu'il faut pour les mettre en place. Pour le moment, les mégadonnées et les approches d'apprentissage machine (ML) qui y sont associées prennent le monde (et certains secteurs agricoles) d'assaut, propulsées par leur court temps de développement et leur précision à la ferme. Il ne fait aucun doute qu'elles auront un rôle majeur à jouer dans l'avenir des systèmes d'aide à la décision en production laitière. Toutefois, leur faible taux d'adoption en production animale jusqu'à présent peut être dû au degré actuel de numérisation, aux fonctionnalités offertes, au rendement de l'investissement ou au défi que représente le maintien d'une technologie délicate dans des milieux corrosifs, poussiéreux et sales. Il est plus que probable que les outils agricoles d'aide à la décision de l'avenir seront une forme hybride de ces deux approches (MM et ML), utilisant les forces respectives de chacune et amplifiant leurs faiblesses. Cet exposé examinera l'utilisation des MM à la ferme et en production laitière de précision et examinera les orientations futures de la production laitière de précision.

The Historical use of Models in Animal Agriculture

Models of all types have a strong history of application in animal production, where their objectives have typically revolved around optimally feeding and growing livestock. For ~50 years, mathematical models have been assisting researchers, nutritionists and producers with decision making on various aspects of livestock production. A typical trajectory for model development has been (1) the execution of experiments to answer targeted questions and generate data, which undoubtedly (2) raise even more questions and then (3) compels scientists to explore ways to summarize and combine this data, and eventually (4) develop mathematical models to solve the problem of the ever-growing body of data and knowledge being generated and make sense of it. As such, these (predominantly nutritional) models have evolved to mathematically express our cumulative knowledge of how a biological system works, developed in order to understand and manipulate nutrient dynamics in the animal and develop better more efficient and productive feeding programs.

Model Types and Classification

Modelling research scientists often talk about models in terms of their classification, or type, as each has relative strengths and weaknesses and niches for application. For example, models are commonly classified as being static or dynamic, deterministic or stochastic, empirical or mechanistic (Thornley and France, 1984). To delve deeper, a static model is one that makes a prediction at a single time point, whereas a dynamic model is one that considers changes in a biological system over time (typically via a series of integrated differential equations or time step loops). A deterministic model considers the 'average' animal and the average animal's response to an intervention, whereas a stochastic model is probabilistic, and models response variation. An empirical model is a model that studies and describes correlations in the data (e.g. $y = mx + b$), vs a mechanistic model is a model that describes an underlying causal pathway. To conceptualize the latter, if a cow is represented by level i , then the organs may be represented by level $i - 1$, cells as level $i - 2$ and the herd by level $i + 1$, and a mechanistic model may predict level i outcomes with a mathematical description of level $i - 1$ (always a level lower) attributes (Thornley and France, 1984).

In practice, the lines drawn between model classifications are blurry at best, as, for example, many mechanistic models contain empirical elements, or a deterministic model may have strategic variation/stochastic attributes introduced (e.g. in inputs), but not throughout. Many of the most advanced models in dairy production are mechanistic or quasi-mechanistic (the latter being a hybridization of the empirical and mechanistic approaches, for example the new INRA feeding system for ruminants, Nozière et al., 2018).

Another way models may be classified is whether they are ‘requirement-based’ or ‘response-based’ models. Requirement based models start with a pre-defined production level, from which they work backwards to determine the nutrient requirements to support that production and then match nutrient supply with requirements. A response model, on the other hand, starts with a description of nutrient supply and the animal, then predicts how the animal will respond to that nutrient supply, and via an optimization routine can find the optimal solution for any objective function (for a nice discussion see Daniel, 2016). While both approaches are useful and applied in practice, a major limitation of the requirement-model approach is that it cannot adapt to the increasing diversity of objectives of dairy production systems (e.g. optimizing vs. maximizing production, feed efficiency, animal health, longevity, etc.). Thus there is a push to increasingly move towards response-based models given their higher level of flexibility.

Ruminant Nutrition Models

Globally, there are numerous groups involved in the development of empirical, quasi-mechanistic and mechanistic models useful for dairy production systems. For an excellent review of the historical evolution of these models, see Tedeschi (2019). In North America, the most commonly used and researched/developed dairy nutrition models include the National Research Council (NRC, 2001), the Cornell Net Carbohydrate and Protein System (CNCPS) (Fox et al., 2004) and Molly (Baldwin, 1995), though other dairy model work exists both within industry and at various academic institutions in North America (for e.g., Dijkstra et al., 1992; Mills et al., 2001; Ellis et al., 2014, as just a few examples). These models have been modified, stepwise, over generations, to account for specific concerns and topics of the era (e.g., performance, efficiency, environmental impact, welfare concerns). The NRC (2001) model is the most commonly used requirement-based model for dairy and could be classified as being quasi-mechanistic, static and deterministic, vs MOLLY (Baldwin, 1995), CNCPS (Fox et al., 2004) and others (Dijkstra et al., 1992; Mills et al., 2001; Ellis et al., 2014) could be classified as being response-models and largely mechanistic, dynamic and deterministic.

Models such as these have been applied over the decades to solve nutritional (Boval et al., 2014; McNamara et al., 2017), whole-farm management (Kebreab et al., 2019) and sustainability (Bannink et al., 2010; Ellis et al., 2011; Gregorini et al., 2013; Van Amburgh et al., 2019) challenges in collaboration with research and extension (many application examples in the literature).

The Role of Mechanistic Models in Dairy Production

Intellectually and academically, mechanistic models (MM) (and to an extent empirical/quasi-mechanistic models) provide animal scientists the opportunity to explore how a biological system works, extract meaningful information from data (e.g., metabolic fluxes from isotope enrichments (France et al., 1999), and increase our understanding of complex biological systems. They are often used to summarize experimental data to derive meaningful parameters used in other applications, for example, fractional rates of rumen degradation (France et al., 2000) or specific rates of mammary cell proliferation (Dijkstra et al., 1997). In research, MMs are excellent tools for identifying areas where scientific knowledge is lacking, or where a hypothesis on the regulation of a system may be wrong. As such, failure of a MM to simulate reality indicates an area where the system has not been appropriately described, and this could be due to a false assumption, a lack of appropriate data or because the level of aggregation at which the model runs is not appropriate for the research question. When models interact iteratively with animal experimentation, MMs assist movement of the whole field forward by increasing our biological knowledge.

In the field, MMs typically serve as ‘decision-support systems’ or ‘opportunity analysis’ tools, and thus go above and beyond least cost diet formulation. Here we might define ‘decision-support’ as the ability to assist with complex (nutritional, management, etc.) decision making, and ‘opportunity analysis’ as the ability to examine a variety of scenarios for their potential outcomes, with the goal of improving performance, reducing cost, and/or minimizing environmental impact (e.g. Ferguson, 2015). Mechanistic models have been used to solve problems such as: (1) identification of performance limiting factors; (2) determination of the optimal nutrient contents of a feed; (3) evaluation of management factors to optimize performance (Ferguson, 2015); (4) the implementation of strategies to reduce nutrient excretion into the environment (Pomar and Remus, 2019); and (5) forecasting outcomes in scenarios not yet seen in practice (Ferguson, 2015).

Limitations of the MM approach revolve around their manual nature, often extensive input requirements and developer/end-user training requirements. From the end-user perspective, these challenges may mean that MMs may not be user-friendly or approachable enough to guarantee user uptake. Use in the field is typically limited to ‘expert users’ (for example within a nutrition company), as opposed to being directly in the hands of producers. This may, however, be on the verge of changing, with increasing use of sensors, technical training and automated data collection on farm.

The Big Data Wave

The term “big data” has gained considerable attention in recent years, though its definition tends to differ across disciplines (Morota et al., 2018). Common themes in ‘big data’ definitions are: (1) *volume*: that the volume of data is so large that visual inspection and processing on a conventional computer is limited; (2) *data types*: may include digital images, on-line and off-line video recordings, environment sensor output, animal biosensor output, sound recordings, other unmanned real-time monitoring systems; and (3) *data velocity*: the speed with which data are produced and analysed, typically in real-time. In order to gain insight from these large volumes of readily available data, it has become increasingly popular to apply data mining and machine

learning (**ML**) methodologies to cluster data, make predictions or forecast in real-time. Thus, the topics ‘big data’ and ML, though not explicitly tied together, often work hand-in-hand.

The emergence of big data and its associated analytics is visible in scientific referencing platforms such as Scopus, where yearly ‘big data’ references rose from 680 in 2012, to 16,562 in 2018. When combined with the keywords ‘cattle’, ‘pigs’ or ‘poultry’, the first reference to ‘big data’ appears in 2011, but there are only 172 total references from 2011-2018 inclusive, indicating a much slower development rate within animal production systems. Liakos et al. (2018) highlighted that 61% of published agriculture sector papers using ML approaches were from the cropping sector, 19% in livestock production and 10% in each of soil and water science, respectively. There may be several reasons for the slower adoption rate in animal production systems, including the current degree of digitalization, the utility offered, low/unclear value proposition, return-on-investment (**ROI**), and the challenge of maintaining sensitive technology in corrosive, dusty and dirty environments.

Machine Learning Models

Artificial intelligence (**AI**) refers to an entire knowledge field focused on the development of computer systems able to perform tasks that normally require human intelligence (e.g. visual perception, speech recognition, decision-making). Machine learning (**ML**), is actually a subgrouping within AI, focused on the development of algorithms and statistical models used to perform specific tasks, without explicit instructions, relying on patterns and inference within the data. This must already sound familiar, and in fact, several ML approaches are already familiar to us – such as linear and nonlinear regression and principal component analysis. Machine learning represents an umbrella term covering numerous empirical modelling methods, including those most common to us.

A broader categorization of ML models (beyond traditional regression), that fits with the scope of big data would be grouping based on the type of learning used therein (supervised, unsupervised), the nature of the data (continuous, discrete) and the category of problems they solve (classification, regression, clustering, dimensionality reduction). In supervised learning, as it pertains to ‘big data’, ML systems are presented with inputs and corresponding outputs, and the objective is to construct a general rule, or model, which maps the inputs to outputs. By ‘learning’, we refer to the ability for the model to improve predictive performance through an iterative process over time on a defined ‘training dataset’. Compared to the MM approach where evaluation of model performance is generally manual, ML systems automate this step and iterate towards the best model. The performance of the ML model in a specific task is defined by a performance metric such as minimizing the residual error or mean square error, the same tools applied within MM evaluations. The goal is that the ML model will be able to predict, classify or reduce the dimensionality of new data using the experience obtained during the training process.

Conversely, in unsupervised learning, there is no distinction between inputs and outputs, and the goal of the learning is to discover groupings in the data. ‘Clustering’ is a type of unsupervised learning problem aimed to find natural groupings, or clusters, within data. Examples of clustering techniques include k-means (Lloyd, 1982), hierarchical clustering (Johnson, 1967) and the expectation maximisation. Dimensionality reduction, another unsupervised learning method, is the

process of reducing the number of variables under consideration by reducing their number to a set of principal variables (components). Principle component analysis (**PCA**) is a common example of a widely applied dimensionality reduction technique.

Machine learning methods have been shown to help solve multidimensional problems with complex structures in the pharmaceutical industry and medicine, as well as in other fields (LeCun et al., 2015). In this respect, they represent a very powerful data synthesis technique. Similarly, Liakos et al. (2018) found in a review that within the agriculture sector, papers using ML approaches largely focused on disease detection and crop yield prediction. The authors reflected that the high uptake in the cropping sector likely reflects the data intense nature of crop production and the extensive use of imaging (spectral, hyperspectral, near-infrared, etc.). Based on a review of the available literature, currently the application ‘niche’ occupied by ML/big data models in animal production revolve around ‘pattern recognition’ (encompassing classification, clustering) and ‘predictive ability’.

Pattern Recognition

Broadly, ML methodologies demonstrate strength interpreting various types of novel data streams (e.g. audio, video, image) to cluster, classify or predict based on supervised or unsupervised approaches and mapping of patterns within the data. Within animal production, this has most notably been applied to animal monitoring and disease detection.

For example, a series of sensor types, with ML models behind them to interpret and classify the data, have been developed for use in practice to monitor changes in animal behaviour (which may signify a change in health status, injury, heat or energy expenditure level) or are used for animal identification. Numerous publications have shown the ability of sensors to classify animal behaviour (grazing, ruminating, resting, walking, etc.), for example via 3-axis accelerometers and magnetometers (Dutta et al., 2015), optical sensors (Pegorini et al., 2015) or depth video cameras (Matthews et al., 2017), along with ML models to classify the collected data. As continuous human observation of livestock to the extent that a subtle change in behaviour could be observed and early intervention applied is often impractical, the niche for automated monitoring systems to track animal movement and behaviour has formed.

As well, several other examples of how big data and ML has been applied to the task of early disease detection in cattle can be found in the literature. For example, Hyde et al. (2020) used a ML model (random forest algorithm) to automate mastitis detection in dairy herds and specify whether the origin was ‘contagious’ or ‘environmental’. Ebrahimi (2019) and Ebrahimie et al. (2018) have demonstrated the use of ML models to detect clinical and sub-clinical mastitis at the individual cow level based on milk-robot information. In other species, researchers have developed ANN models, which analyse poultry vocalizations in order to detect changes and identify suspected disease status earlier than conventionally possible. For example, Sadeghi et al. (2015) recorded broiler vocalizations in healthy and *Clostridium perfringens* infected birds. The authors identified five features (clusters of data) using an ANN model, which showed strong separation between healthy and infected birds, and were able to differentiate between healthy and infected birds with an accuracy of 66.6% on day 2 and 100% on day 8 after infection. Early disease detection seems ideally suited to the ML approach, and is likely to see great success and implementation in this realm.

Predictive Abilities

Data driven methodologies have also found a niche in forecasting and predicting (e.g.) numerical outcomes due to their strong fitting abilities and ability to map even minute levels of variation (e.g. within an ANN). As such, within animal production systems they are well situated to forecast performance metrics of economic importance such as body weight (BW), egg production or milk yield. Alonso et al. (2015) used a Support Vector Machine classification model to forecast the BW of individual cattle, provided the past evolution of the herd BW is known. This approach outperformed individual regressions created for each animal in particular when there were only a few BW measures available and when accurate predictions more than 100 days away were required. Pomar and Remus (2019) and Parsons et al. (2007) as well as White et al. (2004) proposed the use of a visual image analysis system to monitor BW in growing pigs from which they could determine appropriate feed allocations, and this was built into their precision feeding system.

Approach Limitations

Limitations of the ML approach include (1) the risk of over-fitting the data, whereby the ML model learns the ‘noise’ in the data and is unable to make adequate predictions on new data, (2) the data volume requirements are high (ML models are ‘data hungry’) in order to avoid biased and skewed predictions, and (3) the lack of transparency behind each prediction. While the MM is a fully ‘white box’ approach – the reasoning behind predictions is fully visible and the logic can be followed, many ML methodologies are ‘black box’, meaning a prediction is produced, which may in fact be a very good prediction, but a causal explanation or rationale for the prediction is absent.

As another perspective on approach limitations, Rutten et al. (2013), as later summarized by Liebe and White (2019), examined published ‘big data’ analytics used for precision management, and classified them as they fell within four categories, being: (1) techniques, (2) data interpretation, (3) integration of information and (4) decision making. They found that most animal agriculture analytics fell into categories (1) and (2) and that integration of these information ‘tools’ into on-farm decision making systems was largely absent (though we may be on the cusp of this development). Hyde et al., (2020) also commented that despite the large quantity of research into the use of ML technologies to impact (human) clinical management of patients, that examples of translation into an effect on clinical management are seldom found (Clifton et al., 2015). This is an interesting phenomena, which warrants further exploration of the value and barriers to implementation of these new technologies (a few suggestions of which we have provided above), as within animal production systems big data/ML technologies have yet to be widely adopted.

Precision Dairy Nutrition Systems

Precision nutrition is ‘the’ arena where we will see full integration of MM and ML approaches to develop automated knowledge-based nutritional decision-making for dairy. Success in swine (Pomar et al., 2015) and poultry (Zuidhof, 2020) has been demonstrated, and the impact of feeding individuals vs the herd has been explored in dairy (Little et al., 2016; Henriksen et al., 2019).

Within such approaches, big data/ML technologies are involved in the monitoring of individual animals (intake, performance, behaviour, health) and MMs are involved in decision making.

The precision feeding approach has developed because feeding the herd as a group typically implies satisfying the requirements of either (1) the average animal or (2) the highest producing animals, resulting in either (1) half the herd receiving less than their requirements or (2) most individuals receiving more nutrients than required. The latter is often the approach, as for most nutrients, underfed individuals may have reduced performance while overfed individuals may still have near optimal performance. Therefore, providing an excess of nutrients ensures that herd performance is not compromised, though it (negatively) results in economic and environmental waste. As an alternative, precision nutrition targets feeding small-group or individually tailored diets, laying the foundation to address key issues in animal production systems, such as (1) reducing feed cost by improving efficiency, (2) improving sustainability and reducing nutrient excretion into the environment, and (3) improving food safety via traceability (Pomar et al., 2015). Precision nutrition is an innovative approach to livestock production based on advanced on-farm monitoring technologies and our cumulative scientific knowledge of animal science, with the objective of optimizing animal production by controlling variability that exists among farm animals and targeting nutrient delivery to the individual (Pomar et al., 2015). However, nutrient ‘requirements’ and performance vary greatly among individual cows within in a herd, in addition to over time as well (through varying physiological phases, stage of lactation, etc.), and thus knowledge of how individuals will respond, based in biology, is a necessity.

To successfully implement such an (automated) precision nutrition system we require (1) precise knowledge of the individuals (identities, milk yield, milk composition, body weight, body condition score, etc. in real-time), (2) a means to forecast next-day performance, and (3) an integrated decision-making tool to guide what should be fed to each individual based on predicted response to nutrient delivery. With the arrival of the ‘big data wave’ in animal production, item (1) is within reason to be collected on farm daily via sensors. To interpret that data and forecast next day performance (2), ML algorithms are ideally suited to this task based on real-time monitoring of (for example) milk production and bodyweight. Mechanistic models logically lie at the heart of item (3), the decision making ‘engine’ within the system – what should be fed to who? In this fully automated system, it is evident that the two seemingly divergent realms of MM and ML occupy related but synergistic ‘niches’, and it is likely that the future of precision nutrition on farm is a hybridization of these approaches, as has been demonstrated in other species (Pomar et al., 2015).

Gargiulo et al. (2018) conducted a survey in Australia, on the uptake and attitude towards precision dairy technologies. First, they found that farmers with more than 500 cows adopted between 2 and 5 times more specific precision technologies (such as automatic cup removers, automatic milk plant wash systems, electronic cow identification systems and herd management software). They also found only minor differences between how large and small farms felt about the future prospects of precision technologies – both were optimistic. Interestingly though, service providers expected a higher adoption of (for example) automatic milking and walk over weighing systems compared to farmers (60.3 vs. 79.4%, respectively). This difference is likely related to the cost and risk related to implementing said technology, variable on-farm performance and unclear monetary and non-monetary benefits (Jago et al., 2013).

The Future of Precision Nutrition

It is likely that the extent to which farms fully digitalize and become ready to utilize these digital technologies will vary globally. As a result, we may never see precision nutrition implemented in all dairy operations, and instead may observe it on a spectrum, such as:

- 1) No digitalization: Simple empirical models may preside, which can address major issues with easily manipulated equations and minimal input data;
- 2) Manual data pipeline: A MM model with a custom developed front-end may be most suitable;
- 3) A small digital pipeline with a limited number (1 or 2) of data streams: May enable automatic input population for application of MM models;
- 4) A medium digital pipeline: Would mean combining different data sources with management systems delivering real-time data. Some simulation functions of MM may be replaced by real-time variation. The need for heavy-duty front-end development may be reduced.
- 5) A fully digitized pipeline: Would enable MM and ML models to run a farm, monitoring the status and automatically implement generated recommendations (e.g. Tesla's AutoPilot, vertical farming).

Within such a categorical analysis of the spectrum, a complete lack of digitalization (1) might suite the use of a simple model by (for example) a 3rd party consultant, based on rough information provided (from memory or estimation) by the farmer. This scenario is often present in under-developed countries. The development of a manual data pipeline (2) (e.g. manual measuring and recording of data) allows the development of improved 'benchmarking' abilities – and therefore more reliable model predictions from a simple empirical or MM. With a small continuous digital pipeline (3), continuous optimization and real-time benchmarking (as is being achieved now in precision nutrition for swine, e.g. Pomar et al., 2015) becomes possible. It is at this point on the scale of digitalization that things become interesting. From this point forward there also presents a niche for hybridization of the ML and MM approaches, which are described throughout this paper. A medium sized digital pipeline (4) would allow real-time optimization of integral parts of the MM engine, and combine it with digital data. For example, a ration MM-optimized off real-time or ML forecasted intake and BW data, and on the side utilizing a ML model on audio/video data to determine (e.g.) health, activity level or behaviour. A full digital pipeline (5) would allow monitoring and optimization of the entire system. This would allow a MM to be utilized as the basis and optimize/augment with ML learning patterns never envisioned – straight up the ladder of causation (e.g. see Pearl and Mackenzie, 2018). Similar to vertical farming, it would mean the automated management of a farm. To our knowledge, (5) does not yet exist within animal production systems.

As we move towards precision agriculture across species, there may be multiple ways these two modelling approaches will interact. One possible way to achieve this, may be individual parameterization of MMs, via integrating backwards propagation techniques applied within artificial neural networks (ANNs) (for example) into a MM, while placing biological confidence limits around MM parameters permitted to vary between animals to prevent extending beyond biologically sensible values. Knowledge of the biological uncertainty, and setting bounds to limit

purely empirical fitting of the model, may be critical to keep the integrity of the MM. Pomar et al. (2015) proposes a real-time closed feedback system to determine nutrient requirements and subsequent feed intakes for individual pigs by combining both MM and ML. A current challenge in applying existing MM response-based models to feeding individuals is that many are developed on pen-fed or data of means of multiple cows, and response of individuals are likely unique (Daniel, 2016). This may require additional attention as we shift our models from the average cow to the individual.

A diagrammatic of how full integration might generate value is presented in Figure 1. Figure 1 summarizes the flow from a problem statement to data to information to knowledge to wisdom (as illustrated in the data-information-knowledge-wisdom pyramid of Ackoff, 1989) and how MM and ML may assist at different steps of that flow. In general, the ML/big data methods may independently only get us so far as the translation of ‘data’ into ‘information’ (perhaps up until knowledge – e.g. disease detection). The MM methods translate ‘knowledge’ into ‘wisdom’, but may lack sufficient ‘information’. Therefore, their integration would seemingly benefit both realms on the path to ‘explainable AI’.

Another approach may be to ‘train’ the biological knowledge from a MM into a ML model, via, for example, training the ML with a latticework of simulations from a MM. Further, as dairy production increasingly experiences demands to simultaneously optimize to multiple criteria (e.g. performance, cost, efficiency and environment), multi-criteria optimization tied to a MM may be required with the biological knowledge of how these factors interact (e.g. see White and Capper, 2014) – utilizing complex parameter optimization algorithms.

The true challenge to widespread adoption of precision feeding in animal production is likely financial and logistical – requiring a substantial investment in facility re-design and technology upgrade. Not until the ROI can be demonstrated, logistical barriers overcome and trust in the system is developed (there is much at stake within animal production if things go wrong), is precision feeding likely to be taken up by the industry as a solution for the future. However, the potential is there to use precision nutrition and automation of the farm as a means to target nutrient delivery, improve performance and herd efficiency, reduce the environmental impact of dairy production and improve overall animal health and wellbeing.

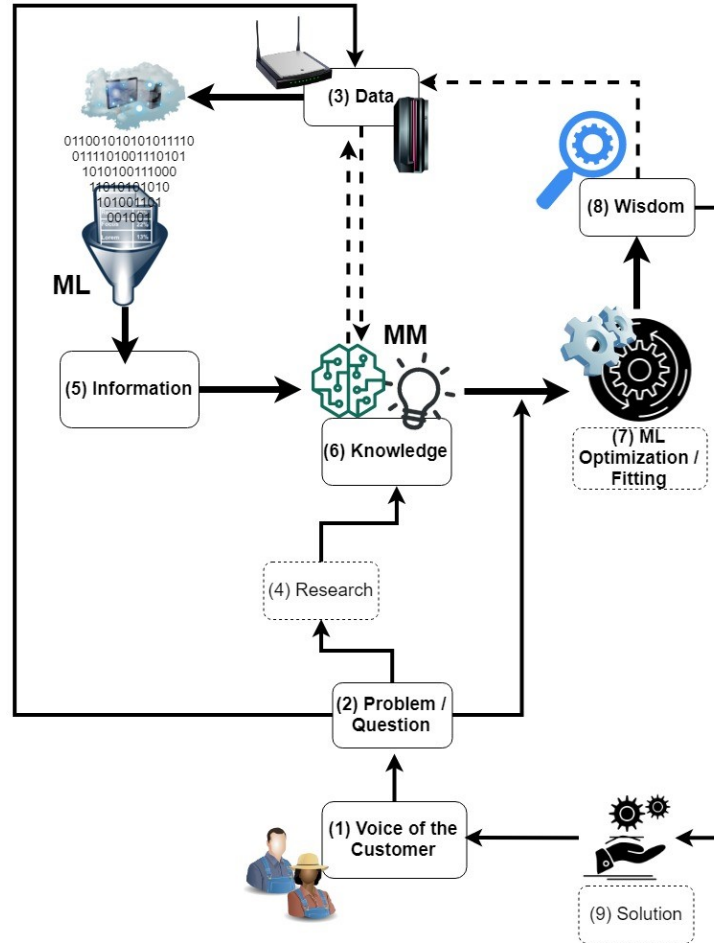


Figure 1. The potential integration of mechanistic models into a decision support system for precision dairy production (Adapted from Ellis et al., 2020).

Summary

Fully automated on-farm precision nutrition is an emerging area within the field of dairy nutrition, targeting the delivery of nutrients to individual animals in order to reduce feed cost, improve feed efficiency and reduce the environmental impact of dairy production. While the concept is not new, the emergence of novel on-farm data streams, allowing producers to follow and monitor the activity, health and productivity of individual animals, is allowing new development in this field. Mechanistic models have served at the heart of nutritional ‘decision-support’ on-farm for decades, and the development of fully automated data collection is opening the door to changes being made on-farm in real-time. The adoption rate of these new technologies is currently relatively slow, and issues such as the current degree of digitalization, access to Wi-Fi on farm, the utility offered, return-on-investment (**ROI**), and/or the challenge of maintaining sensitive technology in corrosive, dusty and dirty environments will need to be addressed. More than likely, the future of on-farm decision support will be a hybridization of MM and ML technologies, utilizing the respective strengths and augmenting weaknesses of each approach.

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Meeting B Vitamin Requirements of Dairy Cows, an Effective Way to Improve Metabolic Efficiency

Comblent les besoins en vitamines B de la vache laitière, un moyen efficace d'améliorer l'efficacité métabolique

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Abstract

One of the major roles of B vitamins is as coenzymes required for enzyme activity and as such B vitamins play critical roles in protein, carbohydrate and lipid metabolism. Consequently, it is likely that the demand for these cofactors increases with milk component yields. Studies conducted more than eight decades ago concluded that the amounts of vitamins provided by the diet and synthesized by the ruminal microflora were sufficient to prevent apparition of deficiency symptoms in mature ruminants. However, apparition of deficiency symptoms is the last stage of the deficiency; deficiency appears as soon as the supply is inferior to the needs leading rapidly to a loss of metabolic efficiency. Reports of beneficial effects of B-vitamin supplementation suggest that, under some conditions, the need for B vitamins exceeds the supply from the diet and the synthesis by rumen microbiota. Given their roles in functioning of enzymes, it is not surprising that an inadequate B-vitamin supply reduces metabolic efficiency by slowing down critical metabolic reactions or by shifting to alternative metabolic pathways with higher energy cost. Responses to B-vitamin supplementation are variable mostly due to the very limited knowledge on dietary factors driving the fate of B vitamins in rumen and consequently, impairing our ability to estimate B vitamin supply. The present paper using information on folates and vitamin B₁₂ metabolism in dairy cow aims to illustrate how, in a perspective of precision farming to optimize metabolic efficiency, meeting dairy cow needs for B vitamins cannot be overlooked.

Résumé

L'un des rôles majeurs des vitamines B est en tant que coenzymes nécessaires à l'activité enzymatique; en tant que telles, les vitamines B jouent un rôle critique dans le métabolisme des protéines, des glucides et des lipides. Par conséquent, il est probable que la demande de ces cofacteurs augmente avec la production de composantes du lait. Des études menées il y a plus de huit décennies ont conclu que les quantités de vitamines B fournies par l'alimentation et synthétisées par la microflore du rumen étaient suffisantes pour empêcher l'apparition de symptômes de carence chez les ruminants adultes. Cependant, l'apparition de symptômes de carence est la dernière étape de la carence; une carence apparaît dès que les apports sont inférieurs aux besoins conduisant rapidement à une perte d'efficacité métabolique. Les effets bénéfiques de

supplémentations en vitamines B décrites dans plusieurs études suggèrent que, sous certaines conditions, le besoin en vitamines B dépasse l'apport via l'alimentation et la synthèse par les microorganismes du rumen. Compte tenu de leur rôle dans le fonctionnement des enzymes, il n'est pas surprenant qu'un apport insuffisant en vitamines B réduise l'efficacité métabolique en ralentissant les réactions métaboliques critiques ou en déviant vers des voies métaboliques alternatives ayant un coût énergétique plus élevé. Les réponses à la supplémentation en vitamines B sont variables, principalement en raison des connaissances très limitées sur les facteurs alimentaires affectant le devenir des vitamines B dans le rumen et, par conséquent, altérant notre capacité à estimer l'apport en ces vitamines. Le présent article utilisant des informations sur le métabolisme des folates et de la vitamine B₁₂ chez la vache laitière vise à illustrer comment, dans une perspective d'élevage de précision pour optimiser l'efficacité métabolique, la satisfaction des besoins des vaches laitières en vitamines B ne peut être négligée.

Introduction

Precision nutrition aims to design nutritional recommendations in order to increase metabolic efficiency, reduce costs, improve product quality, minimize environmental impact while improving the health and well-being of dairy cattle at the individual level. According to Gonzales et al. (2018):

“Precision animal nutrition requires the application of principles, techniques and technologies that automatically integrate biological and physical processes related to animal nutrition using remote monitoring, modelling and control tools that allow making precise, accurate and timely decisions”.

The level of precision achieved is, however, dependent of the understanding of the biological processes involved. Nutrient supply and demand are the main targets to manage but limiting the modelling tools to control energy and major nutrient balance is likely to leave large portions of the variability unacknowledged for. Minor nutrients, such as trace metals and vitamins, are involved in major metabolic pathways, their supply affecting metabolic utilization of energy and major nutrients. Consequently, an inadequate supply of minor nutrients could explain some of the discrepancies between the outcomes of prediction models and observed animal performance under conditions otherwise similar.

Among the minor nutrients required for the physiological functions essential to life are B vitamins. Unlike other nutrients, the vitamins do not serve structural functions, nor does their catabolism provide significant energy. B vitamins play different key roles in metabolism but they all share a common role as coenzymes, metabolites essential for the catalytic activity of enzymes (Combs and McClung, 2017).

In dairy cows, with the exception of vitamin B₁₂, B vitamins are provided by the diet and synthesis in the rumen. Vitamin B₁₂ supply is entirely dependent of its synthesis by the rumen microbiota, if the cobalt supply is sufficient. In mature ruminant, the amount of B vitamins provided by the diet and synthesized by the ruminal microflora is generally sufficient to prevent apparition of deficiency symptoms. Nevertheless, deficiency symptoms are the last stage of the deficiency;

deficiency appears as soon as the supply is inferior to the need leading to a loss of metabolic efficiency (Combs and McClung, 2017). Indeed, lack of a coenzyme would progressively slow down the metabolic reaction and then, drive to a shift towards an alternative pathway not requiring this coenzyme but with a greater energy cost. Beneficial effects of B-vitamin supplementation suggest that, even in mature cows, the need for B vitamins can sometimes exceeds the supply from the diet and the synthesis by rumen microbiota, reducing metabolic efficiency and leading to sub-optimal milk production.

The present paper is not an exhaustive description of the importance of each B vitamin in dairy cow nutrition. Its objective is to illustrate by an example, in the present case, folic acid and vitamin B₁₂, how an inadequate supply of minor nutrients could impact metabolism and lactational performance of dairy cows.

What is the Interest of Folates and Vitamin B₁₂ for Dairy Cow Nutrition?

Metabolic Roles of Folates and Vitamin B₁₂

Folates are enzyme co-substrates for the transfer of one-carbon units obtained from formate or catabolism of amino acids, such as serine, glycine, histidine. The one-carbon units provide through folate metabolism are essential for DNA synthesis and replication, and thus, cell division. Folate metabolism also provides one-carbon units for *de novo* synthesis of methyl groups for formation of the primary methyl donor for biological methylations, S-adenosylmethionine.

S-adenosylmethionine transfers its methyl group in many reactions, among them DNA methylation, which controls gene transcription and genetic stability, and synthesis of phosphatidylcholine, creatine and neurotransmitters (Choi and Mason, 2000).

Folate metabolism plays a major regulatory role in milk protein synthesis in mammary epithelial cells (Menzies et al., 2009a). In many mammal species including dairy cows, it was observed that, during periods of increased milk protein secretion, the greatest change in gene expression in mammary epithelial cells is for the folate receptor alpha (*FOLR1*) which allows the entry of folates into the cells (Ramanathan et al., 2007; Menzies et al., 2009b; Menzies et al., 2009a). These observations were supported by the fact that hormonal stimulation of milk protein synthesis in mammary explants from dairy cows increased expression of *FOLR1* and *ALDH1L1*. The latter enzyme allows the entry of one-carbon units into the folate cycle (Menzies et al., 2009b).

Vitamin B₁₂ acts as coenzyme in only two metabolic reactions. The vitamin is the coenzyme for the cytosolic enzyme, methionine synthase, the critical interface between folates and vitamin B₁₂ which allows the transfer of the methyl group from the 5-methyl-tetrahydrofolate to homocysteine for regeneration of methionine. Besides this role, the vitamin is the coenzyme for the mitochondrial enzyme, methylmalonyl-coenzyme A mutase, essential for the entry of propionate into the Krebs cycle and gluconeogenesis (Combs and McClung, 2017).

Metabolism of these two vitamins is tightly linked. In most mammals, a vitamin B₁₂ deficiency diverts all available one-carbon units towards the synthesis of 5-methyl-tetrahydrofolate, a form of folates which can be demethylated only by the vitamin B₁₂-dependent enzyme, methionine synthase. Consequently, a lack of vitamin B₁₂ leads to a secondary folate deficiency due to the

accumulation of folates under a form, 5-methyl-tetrahydrofolate, which can no longer be used by the cells (Scott, 1999). This link was confirmed in dairy cows during a study looking at the effects of increasing doses of folic acid in the diet, 0, 2 and 4 mg of folic acid per kilogram of body weight given daily to 32 primiparous and 31 multiparous cows from one month before the expected date of calving until 305 d of lactation (Girard and Matte, 1998). Overall, for the complete lactation, supplementary folic acid had no effect on milk production and composition of primiparous cows although milk production decreased linearly with the increase in folic acid supplementation during the first 100 d of lactation, 28.8, 27.0 and 26.6 kg/d for 0, 2 and 4 mg/kg body weight, respectively. In multiparous cows, however, supplementary folic acid was associated with a linear increase in milk production (8284, 8548 and 8953 kg for cows fed diets supplemented with 0, 2, and 4 mg of folic acid/kg of body weight per day, respectively) and milk casein yield during the complete lactation (3 to 305 d after calving). The effect was greater during the first 200 days in milk. In these cows, folic acid supplementation also decreased milk non-protein nitrogen concentration during the first 100 d of lactation supporting the link between folic acid supply and the efficiency of nitrogen utilization for protein synthesis in the mammary gland. A likely explanation for this difference between the response of primiparous and multiparous cows to the folic acid supplementation is the vitamin B₁₂ status of both groups. Overall during the lactation, plasma concentration of vitamin B₁₂ was lower for primiparous cows and this difference was especially marked during the first 8 weeks of lactation when average plasma concentration of the vitamin was around 170 pg/mL in primiparous cows (Girard and Matte, 1999; Figure 1).

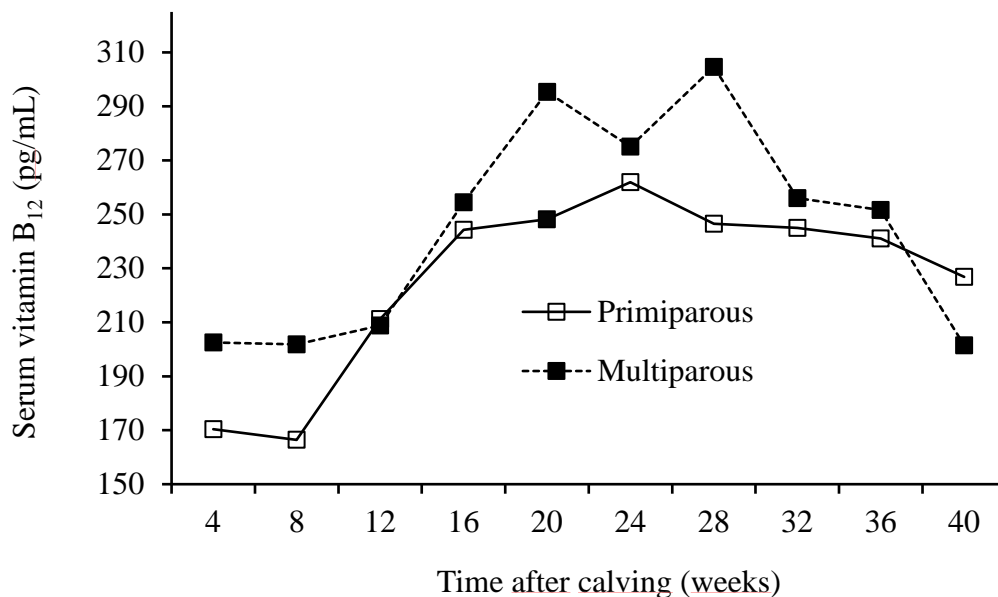


Figure 1. Serum concentration of vitamin B₁₂ during lactation of primiparous and multiparous cows (adapted from Girard and Matte, 1999).

The presence of a secondary folic acid deficiency due to a lack of vitamin B₁₂ was further confirmed by two experiments conducted simultaneously. In the first one, from one month before calving until 305 d of lactation, 54 multiparous Holstein cows were either fed a diet calculated to

supply methionine as 1.75% metabolizable protein, equivalent to 70% of methionine requirement or the same diet supplemented with a rumen-protected methionine supplement to bring the estimated methionine supply to 2.2% of metabolizable protein. Within each diet, the cows received daily 0, 3, or 6 mg/d of folic acid per kg of body weight (Girard et al., 2005). In the second experiment, from the 4th to the 18th weeks of lactation, 14 primiparous Holstein cows were fed the same basal diet than in the first experiment supplemented daily with rumen-protected methionine and 4 mg of folic acid per kg of body weight per day and then, received either a weekly intramuscular injection of saline or 10 mg of vitamin B₁₂ (Girard and Matte, 2005). In the first experiment, there was no effect of supplementary folic acid, methionine or their interactions on milk and milk component yields. It is noteworthy that serum concentration of vitamin B₁₂ was low throughout the lactation but especially so, in early lactation, 181, 232, 257 and 265 at 8, 18, 28 and 38 weeks of lactation, respectively. In the second experiment, in control cows, serum concentration of vitamin B₁₂ was stable throughout the experimental period, under 200 pg/mL. In this experiment, supplementary vitamin B₁₂ increased energy-corrected milk from 25.8 to 29.0 kg/d as compared to cows fed only the dietary supplement of folic acid. Interestingly, packed cell volume and blood hemoglobin were greater when cows received the vitamin B₁₂ supplementation. The increases in packed cell volume and blood hemoglobin concentrations in cows supplemented with folic acid and vitamin B₁₂ suggest that, despite an abundant supply of folates, its utilization for DNA biosynthesis was not optimal under the basal conditions. Moreover, serum methylmalonic acid concentration was lower when cows received the vitamin B₁₂ supplementation. In absence of sufficient vitamin B₁₂ supply, methylmalonyl-CoA from catabolism of propionate accumulates and is degraded in methylmalonic acid (Scott, 1999). Serum methylmalonic acid provides a very sensitive index of vitamin B₁₂ deficiency (Selhub et al., 2009). The decrease in serum concentrations of methylmalonic acid in cows injected with supplementary vitamin B₁₂ further supports the hypothesis that a suboptimal supply in vitamin B₁₂ during early lactation could affect folate utilization and efficiency of energy metabolism as supported by the increased energy-corrected milk yield without changes in dry matter intake.

The opposite situation, a secondary vitamin B₁₂ deficiency due to a lack of folates, has been observed in humans (Selhub et al., 2007) and dairy cows (Graulet et al., 2007; Preynat et al., 2010; Duplessis et al., 2017a). A supplement of folic acid and vitamin B₁₂ increased the affinity of the enzyme, methylmalonylCoA mutase, for vitamin B₁₂ when compared to a supplement of vitamin B₁₂ alone (Graulet et al., 2007). Supplementary vitamin B₁₂ also increased *MUT* expression, the gene responsible for synthesis of methylmalonylCoA mutase, when cows received the folic acid supplement whereas it had no effect when cows did not receive the folic acid supplement (Duplessis et al., 2017a). Similarly, in the study of Preynat et al. (2010), the highest *MUT* expression in liver was obtained with the combined supplement of folic acid and vitamin B₁₂. However, *MUT* expression also tended to be greater in cows receiving only the supplement of folic acid than in control cows. Although folates is not involved in the methylmalonyl-CoA mutase pathway, an adequate supply in folates is essential to the transfer of vitamin B₁₂ from the cytosol to the mitochondria (Riedel et al., 1999).

Transition Period and Early Lactation, Critical Periods for the Folate and Vitamin B₁₂ Balance

As illustrated in Figure 1, the lowest serum concentration of vitamin B₁₂ was observed in early lactation. Colostrogenesis leads to a rapid secretion of large amounts of folates and vitamin B₁₂.

The amounts of folates and vitamin B₁₂ secreted in the first colostrum are 1.5 and 2.6 times the amounts secreted in milk collected at the morning milking during the first weeks of lactation (Table 1; Duplessis et al., 2015).

Table 1. Amounts of folates and vitamin B₁₂ secreted in colostrum and milk of dairy cows fed a dry cow diet providing 1.14 Mcal NE_m/kg DM^a

	Folates (mg)	Vitamin B ₁₂ (µg)
First colostrum	2.9	197
Morning milking, 11 DIM	1.9	75
Morning milking, 39 DIM	2.0	76

^aAdapted from Duplessis et al., 2015

Dietary changes and low dry matter intake during the transition period could reduce folate and vitamin B₁₂ supply during this period of high demand for secretion in colostrum. In addition, due to the metabolic roles of these vitamins, it is to be expected that the consequences of improving metabolic efficiency would be more critical during this period of negative energy and nutrient balance. Consequently, many experiments looking at the effects of supplementing these two vitamins were conducted during the transition period and in early lactation.

Production and Metabolic Responses of Cows to a Combined Supplement of Folic Acid and Vitamin B₁₂

In 7 experiments conducted in cows during the transition period and in early lactation, a combined supplement of folic acid and vitamin B₁₂ had no effect on dry matter intake. The supplement increased milk production in 5 of them (Table 2). Milk total solid, protein, lactose and fat yields were increased by the combined supplement of folic acid and vitamin B₁₂ in 5, 4, 4 and 1 out of these 7 experiments, respectively. Although nutrient composition of the diets fed during these experiments were calculated to meet the NRC (2001) recommendations; the ingredients used, especially forages, differed among experiments.

Table 2. Milk response to a folic acid and vitamin B₁₂ supplement given from 3 weeks before the expected date of calving until 8 weeks of lactation, except for experiment 7 which lasted until 3 weeks of lactation

	EXP 1 ^a	EXP 2 ^b	EXP 3 ^c	EXP 4 ^d	EXP 5 ^e	EXP 6 ^f	EXP 7 ^g
	+	+	+	+	=	=	+
Milk responses, kg	1.4	4.1	2.8	3.6	1.2	-1.7	5.0
<i>P</i> value	0.01	0.01	0.08	0.05	0.29	0.64	0.06

^aGraulet et al., 2007

^bPreynat et al., 2009a

^cPreynat et al., 2009b

^dGhaemialehashemi, 2013

^eGagnon et al., 2015

^fDuplessis et al., 2017

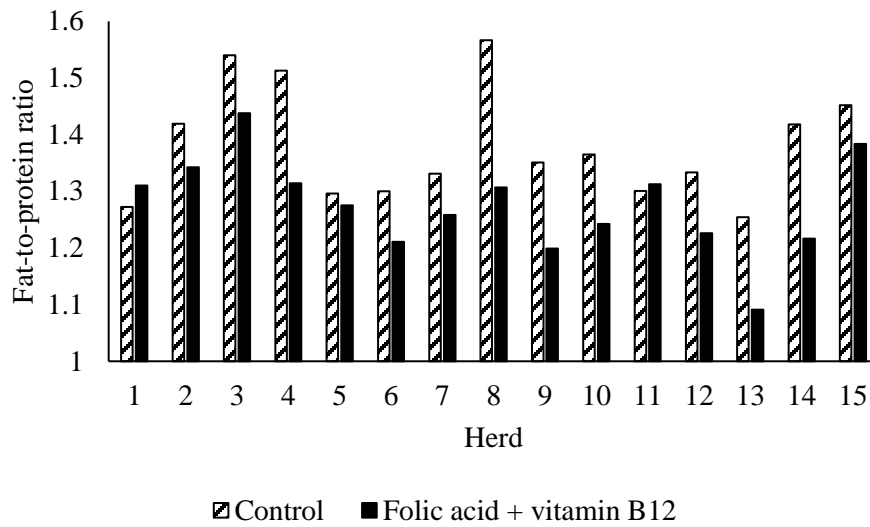
^gDuplessis et al., 2019a; only the first three weeks of lactation

An experiment was also conducted in 15 farms, involving 805 cows. In this experiment, all cows received either weekly intramuscular injections of saline (control) or 320 mg of folic acid and 10 mg of vitamin B₁₂ from 21 days before the expected date of calving until 60 days in milk. Overall, the combined supplement of vitamins increased milk protein content from 30.9 to 31.5 g/kg and decreased milk fat content from 42.1 to 40.3 g/kg, then, decreasing the fat-to-protein ratio. Body condition score and body weight losses were also reduced after calving by the vitamin supplement; during the first 60 d of lactation, body weight losses were 30 and 23 kg for controls and cows injected with vitamins, respectively. The reduction of the fat-to-protein ratio and the body weight losses without effect on milk yield suggest that supplementary folic acid and vitamin B₁₂ improved energy balance in early lactation (Duplessis et al., 2014a). Moreover, in cows at their 2nd lactation or more, the vitamin supplement shortened the interval between calving and 1st insemination by 3.8 days and reduced by 50% calving problems (Duplessis et al., 2014b). Indeed, Gagnon et al. (2015) showed that a combined supplement of folic acid and vitamin B₁₂, at the doses used in the farm trial during the same physiological period, upregulated genes related to follicle differentiation and ovulation suggesting that the supplement promoted a more rapid growth of the dominant follicle. Using an identical experimental design, Ghaemialehashemi (2013) observed that the vitamin supplement increased the number of large follicles and the size of the dominant follicle. He also observed a numerical decrease of the number of days in milk at the first insemination. In both experiments, body reserve mobilization seems to have been reduced by the vitamin supplement without change in dry matter intake (Gagnon et al., 2015; Ghaemialehashemi, 2013).

Variability of Production and Metabolic Responses of Cows to a Combined Supplement of Folic Acid and Vitamin B₁₂

Another interesting observation from the farm trial is the variability of the response to the combined supplement of folic acid and vitamin B₁₂. Variability could have been expected based on results from controlled experiments described previously. However, the farm trial gave us the opportunity to study the responses in cows receiving the same treatments during the same physiological stages but under different management conditions. Figure 2 illustrated the fat-to-protein ratio and body weight loss in early lactation observed in the 15 farms. A lower fat-to-protein ratio following use of the vitamin supplement was observed in 13 farms. Not surprisingly, BW loss differed among farms in control cows, varying from 9 to 40 kg. Nevertheless, in 12 farms out of 15, body weight loss was smaller in cows receiving the vitamin supplement, varying from 0 to 37 kg.

a)



b)

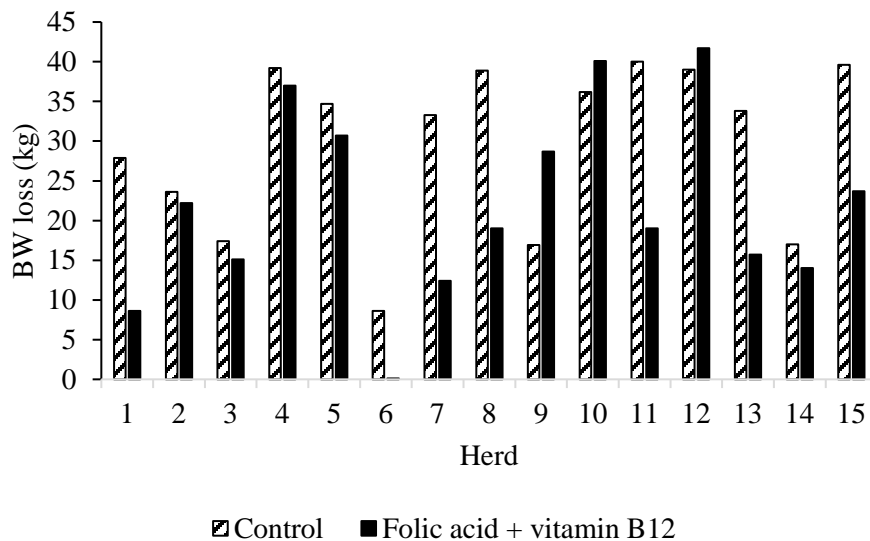


Figure 2. Milk fat-to-protein ratio (a) and body weight loss (b) from calving until 60 days in milk in cows receiving weekly intramuscular injection of saline (control) or 320 mg folic acid and 10 mg of vitamin B₁₂ from 3 weeks before the expected date of calving until 60 days in milk (Adapted from Duplessis et al. 2014a).

Dietary Factors Affecting Folate and Vitamin B₁₂ Status of Dairy Cows

No blood samples were collected to estimate the folate and vitamin B₁₂ status of the cows during this on-farm experiment. However, in control cows, milk concentrations of vitamin B₁₂ varied among farms from 2.25 to 3.84 ng/mL suggesting that the vitamin status differed among farms. Based on diets fed in the 15 farms involved in the experiment, Duplessis et al. (2016) detected a

positive relationship between milk vitamin B₁₂ concentration and dietary acid detergent fiber content and a negative relationship between milk concentration of the vitamin and dietary crude protein content. The former relationship was confirmed by a survey on 100 dairy farms in Québec showing that vitamin B₁₂ concentration in milk was positively related to percentage of fiber (acid detergent and neutral detergent fibers) and negatively related to non-fiber carbohydrate and starch as well as energy of the diet (Duplessis et al., 2019b). Relationships between folate status and dietary factors were not explored in these two studies (Duplessis et al., 2016; 2019b)

A cross-sectional study conducted on Holstein cows at similar stages of lactation across United States and Canada looking at the effect of diet composition on plasma concentrations of folates and vitamin B₁₂ showed that plasma folate concentrations decreased with dietary fiber concentrations (i.e. neutral and acid detergent fibers and lignin) but increased with dietary non-fiber carbohydrate concentrations. The opposite relationships were observed for plasma vitamin B₁₂ concentrations. For example, in this study, the lowest plasma concentration of folates was observed in Californian farms feeding diets with high fiber concentrations whereas the lowest plasma vitamin B₁₂ concentration was reported in Quebec and New York state farms feeding diets with high non-fiber carbohydrate concentrations (Table 3). It is noteworthy, however, that plasma concentrations of folates and vitamin B₁₂ are not highly correlated. Consequently, in spite of this apparent contradiction, a high status in both vitamins can be achieved by feeding diets providing average fiber and non-fiber carbohydrate concentrations as compared to these extremes (Table 3; Duplessis et al., 2020).

Table 3. Average dietary content of fibers and non-fiber carbohydrates (NFC) in areas with different plasma concentrations of folates and vitamin B₁₂^a

Nutrient composition (% of DM)	Highest folate and vitamin B ₁₂ plasma concentrations ^b		Lowest vitamin B ₁₂ plasma concentration ^c		Lowest folate plasma concentration ^d	
	Mean	SD	Mean	SD	Mean	SD
Acid detergent fiber	18.6	3.9	18.0	2.0	23.5	2.0
Neutral detergent fiber	28.1	4.2	30.3	1.3	34.5	3.0
Lignin	3.4	0.8	2.5	0.5	6.2	0.9
Non-fiber carbohydrates	36.1	1.8	42.4	4.3	34.7	3.7

^aAdapted from Duplessis et al., 2020

^bPlasma concentrations of folates and vitamin B₁₂ were 16.6 ng/mL and 249 pg/mL, respectively

^cPlasma concentrations of folates and vitamin B₁₂ were 15.7 ng/mL and 155 pg/mL, respectively

^dPlasma concentrations of folates and vitamin B₁₂ were 11.4 ng/mL and 213 pg/mL, respectively

A meta-analysis on data from 340 lactating cows involved in 16 published studies showed that the amount of vitamin B₁₂ reaching the duodenum, i.e. the amount available for absorption by the cow, is positively related to dry matter intake but inversely related the dietary starch content (Brisson et al., University of Guelph, personal communication). On the other hand, duodenal flow of folates is best predicted by positive relationships with dry matter intake and dietary concentrations of folates and starch (Brisson et al., personal communication). It is noteworthy that in all the studies cited in the present manuscript, cobalt supply was not limiting, it was always at or over the NRC (2001) recommendations.

Differences in diet composition likely entail changes in composition and activity of the rumen microbiota which could alter B-vitamin synthesis and use in the rumen. Magnusdottir et al. (2015) demonstrated that not all bacterial species present in gut are able of *de novo* synthesis of B vitamins; these species are consequently dependent of dietary B-vitamin supply and/or provision by commensal bacteria. Therefore, B-vitamin supply could be modified by alterations of the gut microbiota composition. In dairy cows, results from an observational study support this conclusion. This study showed that abundance of vitamin B₁₂ in the rumen of dairy cow is better linked to the absence of high vitamin B₁₂ consumers than to the presence of efficient vitamin B₁₂ producers. Therefore, to some extent, bacterial consumption of vitamin B₁₂ in the rumen may have a greater influence on the amount of vitamin B₁₂ available for the cow than bacterial production of the vitamin (Franco-Lopez et al., 2020). Knowledge on the relationships between rumen microbiota and B-vitamin supply is extremely limited. This exploratory study highlights the importance of increasing knowledge on rumen microbiota and factors affecting its composition to enable prediction of B-vitamin supply from synthesis in the rumen. Nevertheless, these different approaches help to progressively define dietary conditions to maximize vitamin B₁₂ supply. A similar approach still needs to be conducted for the other B vitamins.

Conclusion

This example supports the concept that, in dairy cows, B-vitamin intake could be insufficient to meet the need. Similar data exist for some other B vitamins (Schwab et al., 2005; Lean et al., 2011). Precision of the nutritional modelling tools is dependent on the acquisition of knowledge on the effects of minor nutrients, such B vitamins, on the efficiency of major metabolic pathways. Given the improvements in production and metabolic efficiency observed when B-vitamin needs are fulfilled, it is clear that controlling energy and major nutrient balance is no longer sufficient to optimise cow nutrition. Precision nutrition is dependent on the ability of the models to evolve taking into account new knowledge. Meanwhile, it is obvious that research is needed to better identify the physiological periods and management conditions leading to a negative balance between B-vitamin supply and metabolic need of the cow and to support the decision of either to use supplements or to change diet composition to avoid deficiency.

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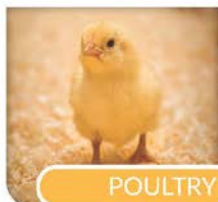


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How the Cow's Daily Pattern of Feed Intake Impacts Milk Synthesis

Synchronisation de la consommation alimentaire pour la synthèse du lait

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Abstract

The dairy cow has a well-recognized natural daily pattern of feed intake and milk synthesis, but regulation of these rhythms and their impact on rumen health and milk synthesis has not been well investigated. Cows consume a large proportion of their daily intake after feed delivery and during the afternoon and early evening. This results in a large dynamic in ruminal fermentation even when feeding a total-mixed ration. Timing feed deliveries to spread intake over more of the day is expected to stabilize rumen fermentation, but modification of the pattern of intake has limitations. There is also a daily pattern to milk synthesis with highest milk yield and lowest milk fat and protein concentration generally observed during the first part of the day. The daily pattern of milk synthesis is dependent on the timing of feed intake and the rhythm can be modified by the frequency of feeding and timing of feed availability. This rhythm is likely partially due to variation in the amount of nutrients available for milk synthesis over the day, but we have also observed modification of mammary circadian timekeepers that regulate tissue metabolism. We expect that maximal milk yield and efficiency are achieved when we have more consistent rumen fermentation and match the timing of nutrient absorption and mammary capacity for milk synthesis. Considering the timing and frequency of feeding while monitoring cow behavior is currently our best intervention.

Résumé

Chez la vache laitière, les rythmes quotidiens naturels de consommation alimentaire et de synthèse du lait sont bien connus, mais leur régulation et leur impact sur la santé du rumen et la synthèse du lait n'ont pas été étudiés en profondeur. Les vaches consomment une grande partie de leur ration quotidienne après la distribution des aliments ainsi qu'en après-midi et en début de soirée. Cela se traduit par de forts effets dynamiques sur la fermentation ruminale, même avec une ration totale mélangée. Répartir la distribution des aliments sur une plus grande partie de la journée devrait stabiliser la fermentation dans le rumen, mais la modification du rythme de consommation a ses limites. Il existe également un rythme quotidien de synthèse du lait, c'est-à-dire que c'est dans la première partie de la journée que la vache connaît généralement le rendement laitier le plus élevé et la concentration en matières grasses et en protéines du lait la plus faible. Le rythme quotidien de synthèse du lait dépend du moment de la prise d'aliments et peut être modifié par la fréquence des repas ainsi que le moment de la disponibilité des aliments. Ce rythme dépend probablement

en partie de la variation de la quantité de nutriments disponibles pour la synthèse du lait au cours de la journée, mais nous avons également observé une modification des chronomètres circadiens mammaires qui régulent le métabolisme des tissus. Nous pensons que le pic de rendement et d'efficacité est atteint lorsque la fermentation ruminale est plus régulière et correspond au moment de l'absorption des nutriments et à la capacité de synthèse du lait des tissus mammaires. Notre meilleure intervention consiste actuellement à tenir compte du moment et de la fréquence de l'alimentation tout en surveillant le comportement des vaches.

Introduction

Biological rhythms are repeating patterns that are driven by time-keeping mechanisms within the animal and are adaptive as they coordinate physiology and metabolism with the external environment. The dairy cow has a well-recognized natural daily pattern of feed intake and milk synthesis and an annual rhythm of milk composition, but regulation of these rhythms has not been well described in the literature or well considered in current dairy management. We commonly assume that feeding a total mixed ration creates constant ruminal conditions, but the large variation in the rate of feed intake across the day causes large fluctuations in rumen fermentation and absorbed nutrients. Milk composition also differs across the day due to both dynamics in nutrient absorption and biological regulation attempting to match milk yield and composition with calf requirements across the day. Managing feeding times provides the opportunity to modify feed intake across the day, but behavior responses are complex.

Background

Rather than simply responding to an environmental stimulus, endogenous timekeepers in the hypothalamus allow the animal to anticipate daily and yearly environmental changes before they occur. The timekeepers create rhythms that then drives adaptive changes in metabolism and physiology that increase survival. Two important aspects are the timing of the rhythms are set or “entrained” by environmental signals, such as light dark cycles, and the rhythms will persist if the animal is held under constant conditions because it is running within the body. Two major rhythms of importance to the dairy cow are circadian and annual rhythms.

Circadian Rhythms

Circadian rhythms refer to 24-hour repeating cycles followed by most physiological functions. Circadian rhythms are created by endogenous timekeeping mechanisms and are adaptive as they temporally coordinate behaviors and physiological processes with daily changes in the environment. Anyone who has flown across time zones or lost a night of sleep, or even just changed clocks to daylight savings time, appreciates the physiological and psychological importance of circadian rhythms. Their importance is also strongly supported by scientific evidence. For example, epidemiological data in humans clearly shows that disruption of circadian rhythms by night-shift work increases mortality and morbidity and is especially associated with many conditions normally associated with stress.

The “biological clocks” that keep track of what time it is exist in most tissues in the body. The biological clocks in metabolically important tissues (e.g. adipose and liver) are responsive both to timing of light-dark cycles that controls the master clock in the brain, but also the timing of food availability. Interestingly, in experimental models the timing of food intake can alter the synchrony between the central master timekeeper and peripheral clocks, resulting in development of numerous disorders including obesity, insulin resistance, and metabolic diseases (Reviewed by Takahashi et al., 2008). We have demonstrated that there is a biological clock in the mammary gland that responsive to the timing of feed intake.

Daily pattern of feed intake

Feeding behavior is centrally regulated through integration of many factors including hunger, satiety, physiological state, environment, and endogenous circadian rhythms (Allen et al., 2005). Grazing cows have a well described “crepuscular” feeding pattern with a large proportion of intake consumed at dawn and dusk (Reviewed by Albright, 1993). It is important to remember ruminants are prey animals and daily feeding patterns are expected to have been impacted evolutionarily by changes in risk of predators and nutritional value of forages over the day. Importantly, pasture forages are highest in sugar and amino acids in the afternoon after photosynthesis has occurred. A circadian rhythm of intake with greater intake during the afternoon synchronizes hunger with maximal forage quality.

Using an automated observation system, we have observed the effect of feeding time and diet composition on the daily rhythm of intake. The daily pattern of intake in high producing cows and the effect of feeding time is well illustrated in an experiment where we fed cows 1x/d at 0830 h or 2030 h (Niu et al., 2014). Over 20 and 34% of daily intake was consumed in the 2 h after feeding in cows fed at 0830 and 2030 h, respectively. The intake rate at other times of day did not differ greatly, with both groups having lower intake overnight and higher intake in the afternoon. Before this work we commonly thought that cows consumed feed mostly during the day because that is when we delivered feed and it was the freshest. Delivery of fresh feed is a strong stimulus for feed intake. However, it is interesting to note that cows fed in the evening had low intake during the overnight (not different from morning fed cows) and waited till the following afternoon when feed was over 16 h old to increase intake to about twice that of the overnight period. This experiment highlights that cows have a strong natural drive to consume feed during the afternoon and early evening and timing of feed delivery is a strong stimulant to modify this pattern and has been replicated in other studies.

Physiological significance of the circadian pattern of intake

The ruminant has a rather consistent absorption of nutrients over the day because of more frequent meals, the size of the rumen, and the slow rate of ruminal digestion. However, highly fermentable diets are commonly fed to maximize energy intake and microbial protein production and result in a rapid production of volatile fatty acids (VFA) after consumption (Allen, 1997). Additionally, differences in the rate of feed intake over the day results in a large difference in the amount of fermentable substrate entering the rumen over the day

The dynamic nature of rumen fermentation throughout the day is supported by high resolution observations of rumen pH by our lab and others (e.g. Yang and Beauchemin, 2006, DeVries et al., 2007, Harvatine, 2012), which clearly show a daily pattern of rumen pH with a nadir approximately

10 h after feeding. We also observed that ruminal digesta weight and starch concentration were 24% and 87% higher, respectively, 4 h after feeding compared to 1.5 before feeding. Additionally, we have observed that ruminal starch and NDF concentration over the day fit a cosine function with a 24 h period demonstrating a daily rhythm (Ying et al., 2015). We are not aware of a characterization of the rate or composition of duodenal flow throughout the day, but a daily rhythm has also been reported for fecal particle size, neutral detergent fiber (NDF), indigestible NDF, and starch concentration (Maulfair et al., 2011). We have also observed that the rhythm of fecal NDF was dependent on the time of feeding (Niu et al., 2014). Taken together, there is strong support for a circadian rhythm of nutrient absorption.

Evidence of circadian regulation of milk synthesis

Dairymen commonly recognize that morning and evening milking differ in milk yield and composition. Quist et al. (2008) conducted a survey of the milking-to-milking variation in milk yield and composition on 16 dairy farms. Milk yield and milk fat concentration showed a clear repeated daily pattern over the 5 days of observation in herds that milked 2 and 3 x/d. We have also observed milk yield and milk composition at each milking while milking every 6 h and feeding cows 1 x/d at 0800 h or in 4 equal feedings every 6 h (Rottman et al., 2014). This demonstrated the daily pattern of milk synthesis in cows and identified an interaction with the timing of feed intake. We have further demonstrated shifts in the timing of milk synthesis through fasting cows for a short period during the day compared to the night (Salfer and Harvatine, 2020).

Recent work at Purdue tested the effect of light-dark phase shifting on metabolic health in transition dairy cows (Suarez-Trujillo et al., 2020). They observed that light phase shifting reduced the circadian rhythms of core body temperature and melatonin. Phase shifted cows also had increased total resting time, but decreased resting bout durations. Phase shifted cows did increase milk yield 2.8 kg/d over the first 60 d of lactation, although this may be due to a change in nutrient partitioning and the long-term effect was not investigated.

Lastly, automated milking systems (AMS) provide an opportunity to observe a natural preference for milking time. Care is needed in interpretation of cow behavior in AMS because of the confounding factors of demand for the robot and the entrainment by multiple factors. However, the frequency of cows entering the milking system appears to follow a circadian pattern (e.g. Hogeveen et al., 2001, Wagner-Storch and Palmer, 2003). For example, Wagner-Storch et al. (2003) reported 2% of cows in the holding area between 0000 and 0500 h compared to 8 to 12% of cow between 0800 and 1900 h. The preference for milking time may be due to a natural circadian synchronization with environmental factors or simply support a natural low activity period of the day.

Effects of Photoperiod on Milk Production

Extensive research has examined the impact of altering photoperiod length on milk synthesis of the dairy cow. The first report of increased milk production after 16 h light: 8 h dark (16L:8D) photoperiod was made by Dr. Tucker's lab at Michigan State (Peters et al., 1978). Since this initial discovery, several subsequent experiments have confirmed these findings (Dahl et al., 2000, Dahl et al., 2012). The effect occurs after implementation of any photoperiod greater than

12L: 12D, however the response is greatest at 16L: 8D. The mechanism of long day lighting on milk synthesis is likely through the same molecular mechanism regulating other circadian rhythms in the dairy cow.

Take Home Messages

- “Biological clocks” within the cow are keeping track of what time of day it is and create daily rhythms.
- This robust system coordinates physiology and metabolism with the external environment and likely helps the cow optimally produce milk while adjusting to changing conditions across the day.
- The dairy cow has a clear daily pattern of feed intake and milk synthesis. The timing of feed intake has a large impact on rumen environment including pH.
- The timing of feed delivery and feed management are our best opportunities to modify the daily pattern of feed intake with the goal of spreading feed intake across a larger part of the day.

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The Effects of Supplementing Low-Complexity Deoxynivalenol-Contaminated Nursery Diets with NutraMix™ or Fish Oil on Growth Performance and Immune Function

Effets de la supplémentation avec NutraMix^{MC} ou de l'huile de poisson des rations de pouponnière peu complexes contaminées par le désoxynivalénol sur la performance de croissance et la fonction immunitaire

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Abstract

It is standard to feed newly weaned pigs high-complexity nursery diets to combat the growth lag induced by stressors of the weaning process. These nursery diets contain multiple sources of highly digestible animal-based proteins, making them the most expensive diets in the production cycle. Pigs can accelerate growth following a period of reduced growth caused by nutritional challenge (including the use of plant-based protein sources), which is known as compensatory growth. Feeding low complexity nursery diets containing increased inclusion of plant-based ingredients may be a means to decrease nursery feed costs, but with greater risk for mycotoxin contamination, which could inhibit compensatory growth. The objective was to evaluate the effects of deoxynivalenol (**DON**)-contaminated low-complexity diets supplemented with NutraMix™ or fish oil on growth performance and immune response. 320 newly weaned pigs were assigned to either a [1] high-complexity, [2] low-complexity or [3] DON-contaminated (3 ppm) low-complexity diet without additive, [4] with NutraMix™ (0.2%) or [5] fish oil (2.5%) for 3 weeks. Thereafter, an *E. coli* lipopolysaccharide (**LPS**) challenge was performed, and the remaining pigs were fed a common, uncontaminated diet for 3 additional weeks. Though DON-contaminated low-complexity diets initially reduced growth performance, all pigs achieved the same final body weight (26.1 kg) following the recovery period. During the LPS challenge, pigs fed NutraMix™-supplemented diets had increased relative liver weights and improved interferon- γ response compared to pigs fed the other DON-contaminated diets. Therefore, nursery pigs can express compensatory growth after exposure to diets containing 3 ppm DON. This indicates that low complexity diets could be used to reduce feed costs, even when DON is present in cereal grains. NutraMix™ may improve the immune response during a disease challenge and when feeding DON-contaminated low-complexity diets.

Key words: Nursery pigs, compensatory growth, deoxynivalenol

Résumé

Il est courant de nourrir les porcs nouvellement sevrés avec des rations de pouponnière très complexes pour combattre le retard de croissance causé par les facteurs de stress liés au processus de sevrage. Ces rations de pouponnière contiennent de nombreuses sources de protéines animales hautement digestibles, ce qui en fait les rations les plus coûteuses du cycle de production. Les porcs peuvent accélérer leur croissance après une période de ralentissement liée à un obstacle nutritionnel (y compris l'utilisation de sources de protéines d'origine végétale); c'est ce que l'on appelle la croissance compensatoire. L'utilisation de rations de pouponnière peu complexes contenant davantage d'ingrédients d'origine végétale peut être un moyen de réduire les coûts d'alimentation en pouponnière, mais elle comporte un plus grand risque de contamination par les mycotoxines, ce qui pourrait inhiber la croissance compensatoire. L'objectif était d'évaluer les effets de rations peu complexes contaminées par le désoxynivalénol (**DON**) et additionnées de NutraMix^{MC} ou d'huile de poisson sur les performances de croissance et la réponse immunitaire. Trois cent-vingt porcelets nouvellement sevrés ont été affectés soit à une ration [1] très complexe, [2] peu complexe ou [3] peu complexe et contaminée par le DON (3 ppm) sans additif, [4] avec NutraMix^{MC} (0,2 %) ou [5] de l'huile de poisson (2,5 %) pendant 3 semaines. Ensuite, les animaux ont été provoqués avec un lipopolysaccharide (LPS) d'*E. coli*, et les autres porcs ont reçu une ration commune non contaminée pendant 3 semaines additionnelles. Bien que les rations peu complexes contaminées par le DON aient initialement réduit les performances de croissance, tous les porcs ont atteint le même poids corporel final (26,1 kg) après la période de récupération. Au cours de la provocation au LPS, les porcs nourris avec les rations additionnées de NutraMix^{MC} ont présenté un foie relativement plus lourd et une réponse à l'interféron- γ relativement meilleure, comparativement aux porcs nourris avec les autres rations contaminées par le DON. Par conséquent, les porcs en pouponnière peuvent afficher une croissance compensatoire après avoir consommé des rations contenant 3 ppm de DON. Ces résultats indiquent que des rations peu complexes pourraient être utilisées pour réduire les coûts d'alimentation, même si les céréales contiennent du DON. NutraMix^{MC} peut améliorer la réponse immunitaire dans un contexte d'exposition à une maladie et de consommation d'une ration peu complexe contaminée par le DON.

Introduction

Nursery pig diets typically contain highly digestible and expensive ingredients (e.g., animal protein sources) to combat the post-weaning growth lag (Campbell and Dunkin, 1983; Lallès et al., 2004). However, after an initial reduction in growth performance, nursery pigs fed simple, corn-and-soybean meal (**SBM**)-based diets can achieve body weights not different from pigs fed nursery diets that contain highly digestible ingredients via compensatory growth (Skinner et al., 2014). This phenomenon could allow for an increased inclusion of plant-based ingredients in nursery diets and to that end, lower feed costs. Soy proteins are also known to induce transient allergic and inflammatory responses (Chen et al., 2011), which may compromise immune function of nursery pigs. Moreover, increasing the inclusion of plant-based ingredients also increases the risk of mycotoxin contamination in nursery diets. Deoxynivalenol (**DON**) is a common mycotoxin that infests cereal grains (Rodrigues and Naehrer, 2012). When present at > 1 ppm in swine rations, DON has been linked to reduced growth performance and impaired immune function (Rotter et

al., 1994) and may interfere with the expression of compensatory growth. Immune modulating feed additives (i.e. blends of antioxidants, preservatives, and amino acids and omega-3 fatty acids) could rescue pig growth performance and immune function in instances of dietary DON contamination (Van Le Thanh et al., 2015; Jin et al., 2017; Huber et al., 2018), which could facilitate compensatory growth, even when DON is present in simple (low-complexity) nursery diets. Therefore, the objective of this study was to evaluate the effects of supplementing low-complexity DON-contaminated nursery diets with immune-modulating feed additives on growth performance and immune function.

Methodology

Three hundred and twenty newly weaned pigs (6.7 ± 0.3 kg BW) were divided into 40 pens and randomly assigned 1 of 5 dietary treatments ($n = 8$): [1] a high-complexity diet containing multiple animal protein sources (**HC**) or 1 of 4 low-complexity diets containing only plant-based proteins with [2] no DON contamination (**LC**), or with 3 ppm DON contamination [3] without feed additives (**DON-**), [4] with NutraMix™ (2 g/kg; **DONNM**) or [5] with fish oil (2.5%, as-fed; **DON ω 3**). Dietary treatments were fed for phases I and II for 7 and 15 days, respectively. Phase III was a common corn-SBM diet which was fed for the subsequent 20 days (recovery phase). Individual pig body weight and per-pen feed disappearance were recorded weekly. After phase II (day 22), an *E. coli* lipopolysaccharide (**LPS**) challenge was performed where 2 pigs per pen were injected with 30 μ g/kg BW of LPS and 1 pig with 1 mL saline. Rectal temperatures were collected at 0, 1, 2, and 3 hours post-injection. Thereafter, plasma was collected for cytokine analysis.

Results

In phase I, ADG, ADFI and G:F were not different for pigs fed the HC and LC diets (Table 1), but were lower for both DON additive treatments ($P < 0.05$); intermediate values were observed for DON-. In phase II, pigs fed the DON- and DON ω 3 diets had lower ADG than LC ($P < 0.05$), lower ADFI than HC ($P < 0.05$), but greater G:F than those fed HC ($P < 0.05$). By the end of phase II (end of the treatment phase), the BW of pigs fed the HC and LC diets were not different, pigs fed DON ω 3 tended to have lower BW than those fed HC and LC ($P = 0.084$ and 0.079 , respectively), and pigs fed DON- and DONNM had intermediate BW. In phase III and over the entire nursery period, there were no treatment effects on ADG, ADFI, G:F or final BW. The LPS challenge induced an immune response, marked by an increase in rectal temperature between 0 and 3 hours after injection (39.9 vs. 40.8°C ; $P < 0.001$), which was not influenced by dietary treatment. The LPS injection increased plasma interferon- γ (**IFN- γ**) concentrations for pigs fed DONNM, HC, and LC diets (Figure 1). However, plasma concentrations of IFN- γ were not different between the saline- and LPS-injected pigs fed the DON- and DON ω 3 diets, since the baseline (i.e. of saline-injected pigs) plasma IFN- γ concentrations were already approximately twice that of saline-injected pigs fed HC, LC, and DONNM diets. Additionally, the IFN- γ response to LPS for pigs fed the DON ω 3 diets was ~30% less than pigs that received the other dietary treatments.

Conclusion

Nursery pigs expressed compensatory growth after exposure to low complexity nursery diets contaminated with 3 ppm DON following a recovery period. Therefore, even in times of moderate DON contamination of cereal grains, low complexity nursery diets could be used to decrease production costs, though immune-modulating feed additives may provide benefits during instances of additional immune system stimulation.

Table 1. Effect of DON-contaminated nursery diets supplemented with NutraMix™ or fish oil on pig growth performance during the nursery period

Item	Dietary treatments					SEM	P-value Treatment
	HC	LC	DON				
			-	NM	ω3		
No.	8	8	8	8	8		
Initial BW, kg	6.7	6.7	6.7	6.7	6.7	0.3	0.996
Phase II BW, kg	13.4 ^x	13.4 ^x	12.7 ^{x,y}	12.9 ^{x,y}	12.6 ^y	0.4	0.018
Final BW, kg	26.5	26.1	25.4	26.1	26.3	0.7	0.523
ADG, g/d							
Phase I	68 ^a	53 ^{a,b}	33 ^{b,c}	20 ^c	17 ^c	12	<0.001
Phase II	406 ^{a,b}	410 ^a	374 ^b	392 ^{a,b}	376 ^b	11	0.006
Phase III	668	664	655	679	698	22	0.232
Overall	472	464	445	462	465	12	0.384
ADFI, g/d							
Phase I	162 ^a	145 ^{a,b}	122 ^{b,c}	115 ^c	107 ^c	9	<0.001
Phase II	519 ^a	500 ^{a,b}	451 ^b	479 ^{a,b}	453 ^b	16	0.005
Phase III	1065	1046	1023	1089	1076	43	0.527
Overall	639	620	584	624	605	22	0.167
G:F							
Phase I	0.41 ^a	0.35 ^a	0.27 ^{a,b}	0.14 ^b	0.14 ^b	0.09	<0.001
Phase II	0.78 ^b	0.82 ^{a,b}	0.83 ^a	0.82 ^{a,b}	0.83 ^a	0.01	0.027
Phase III	0.63	0.64	0.64	0.62	0.65	0.01	0.291
Overall	0.74	0.75	0.76	0.74	0.77	0.01	0.207

^{a,b,c}Means differ within the row ($P < 0.05$)

^{x,y}Means tend to differ within the row ($0.05 \leq P \leq 0.10$)

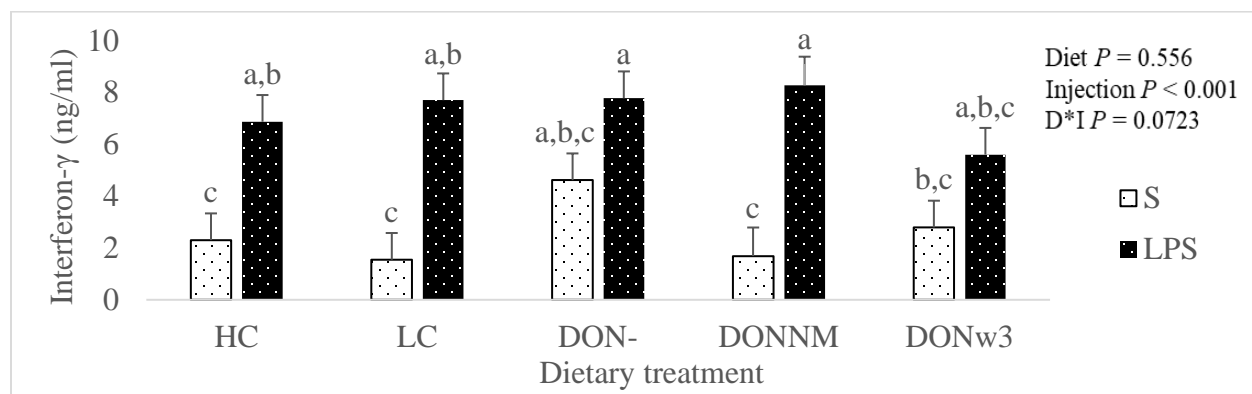


Figure 1. Effect of DON-contaminated nursery diets supplemented with NutraMix™ or fish oil on plasma IFN- γ concentration of pigs injected with LPS or saline at day 22 post-weaning

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Zinc Replacement Strategies for Preventing Piglet Diarrhea

Stratégies de substitution du zinc dans la prévention de la diarrhée chez le porcelet

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Abstract

Several feed additives are proposed as potential substitutes for zinc oxide (ZnO) to reduce incidence of post-weaning diarrhea (PWD) and provide protection against intestinal injury. Any reduction of dietary zinc will result in less excretion of zinc to the feces, and thereby in reduced contamination of the agricultural soil and antimicrobial resistance. Contrary to medical drugs, feed additives apply to improve the performance of healthy animals or to cover their physiological requirements. This presentation aims to evaluate the potential of commonly suggested feed additives for their effect on improving gut health, and in that way reducing PWD. Although the mechanisms behind the success of high dietary ZnO levels in terms of limiting PWD is still not clear, it seems that ZnO has a positive impact on the stability and diversity of the gastrointestinal microbiota, which contributes to an increased colonization resistance against pathogens, as well as an indirect effect on host-immunity and epithelial barrier function. While many potential feed additives share several of the mechanisms by which ZnO is proposed to prevent PWD, there is probably not a single feed additive, which can replace ZnO for its capability to prevent diarrhea. Thus, the ideal feed additive for enhancing gut health of pigs is probably a cocktail of various antibacterial and immunomodulatory agents targeting specific challenges as *E. coli* infection progresses in piglets post weaning, and they may be applied early in life in order to efficiently influence in the established matrix interaction between the microbiota and the host.

Résumé

Plusieurs additifs alimentaires sont proposés comme substituts potentiels à l'oxyde de zinc (ZnO) pour réduire l'incidence de la diarrhée post-sevrage (PWD) et fournir une protection contre les lésions intestinales. Toute réduction du zinc dans l'alimentation entraînera une diminution de l'excrétion de cet élément dans les matières fécales et, donc, une réduction de la contamination des sols agricoles et de la résistance aux antimicrobiens. Contrairement aux médicaments, les additifs alimentaires servent à améliorer les performances des animaux en bonne santé ou à combler leurs besoins physiologiques. Cette présentation vise à évaluer le potentiel des additifs alimentaires couramment employés pour leur effet bénéfique sur la santé intestinale et, par conséquent, sur la réduction de la PWD. Bien que les mécanismes qui expliquent le succès des taux élevés de ZnO alimentaire pour limiter la PWD ne soient pas encore clairs, il semble que le ZnO ait un effet positif sur la stabilité et la diversité du microbiote gastro-intestinal, ce qui contribue à une meilleure résistance à la colonisation par les agents pathogènes et agit indirectement sur l'immunité de l'hôte.

et la fonction de barrière épithéliale. Même si de nombreux additifs alimentaires potentiels partagent plusieurs des mécanismes grâce auxquels le ZnO prévient la PWD, il n'existe probablement pas un additif alimentaire qui puisse à lui seul remplacer le ZnO par sa capacité à prévenir la diarrhée. Ainsi, l'additif alimentaire idéal pour améliorer la santé intestinale des porcs est probablement un cocktail de divers agents antibactériens et immunomodulateurs ciblant des éléments spécifiques à mesure que l'infection par *E. coli* progresse chez les porcelets sevrés et qui peuvent être utilisés en bas âge afin d'influencer efficacement l'ensemble des interactions entre le microbiote et l'hôte.

Introduction

Post-weaning diarrhoea (PWD) is a significant enteric disease causing considerable economic losses for the pig industry. Among several etiological risk factors, enterotoxigenic *Escherichia coli* (ETEC) is considered to be a major cause, i.e. colibacillosis. The use of antibiotics at sub-therapeutic concentrations was routinely used as growth promoters for several decades, but has since 1 January 2006 been banned in the European Union due to the increasing prevalence of resistance to antibiotics in pigs. The removal of in-feed antibiotics from piglet diets has negative economic consequences as it dramatically increases the rate of morbidity and mortality due to ETEC as well as the use of antibiotics for therapeutic purposes. The probably most used substitutes for in-feed antibiotics is the pharmacological levels (at 2,000 to 4,000 mg/kg) of zinc oxide (ZnO) during the first two weeks post-weaning to alleviate the diarrhea and growth check in post weaning piglets in many pig producing countries. Any reduction of dietary zinc will result in less excretion of zinc to the feces, and thereby in reduced contamination of the agricultural soil. Thus, alternatives to antibiotics and to the high levels of ZnO that can control ETEC infections in piglets post-weaning will be of great advantage, also because of the risk of cross resistance between antibiotics and Zn or other heavy metals. A number of nutritional, genetic and management strategies have been reported in the literature as alternatives to in-feed antibiotics to prevent PWD, but, in most studies, the reduction of PWD has not been addressed as a primary response parameter (Lauridsen et al., 2017). The aim of this presentation is to evaluate the potential of commonly suggested dietary strategies for their effect on improving gut health, and thereby, for reducing post-weaning diarrhea (PWD) in pigs.

Pathogenesis of ETEC-post weaning diarrhea and its prevention by ZnO

Post-weaning diarrhea is a multifactorial disease, and its pathogenesis is still unclear. Frequently described, this is a condition of weaned pigs characterized by frequent discharge of watery faeces during the first two-three weeks after weaning. This condition is typically associated with faecal shedding of haemolytic enterotoxigenic *E. coli* (ETEC), proliferating in the ileum. The pathogenesis of ETEC-PWD can briefly be described as follows: 1) adherence of ETEC to specific receptors in the small intestine, 2) ETEC proliferation, 3) production of enterotoxins, and 4) hypersecretion of water and electrolytes to the lumen of the small intestine. The weaning process itself may due to external factors such as dietary changes, antibiotic use, and stressors, trigger inflammation and oxidative stress in the piglet gut, and this leads to a number of changes in the gut environment that favor the blooming of *E. coli*. An obvious solution to the treatment of PWD may therefore be the prevention of enteric inflammation and oxidative stress during the post

weaning period. Other pathogenic bacteria may also be the cause of the PWD, but the presence of pathogens may in fact not necessarily be the cause of diarrhea in pigs. However, during the initial two weeks post weaning, where medical zinc is permitted for treating the PWD, the most frequent cause of diarrhea in pigs is the ETEC, and one may ask why ZnO has been identified as the most efficient alternative to antibiotics for the treatment of PWD in pigs.

Oral zinc supplementation is used for treating diarrhea in children in developing countries. A recent review concluded that in areas where the prevalence of zinc deficiency or the prevalence of malnutrition is high, Zn may be of benefit in children aged six months or more, while no benefit was obtained in children being less than 6 months and well-nourished (Lazzerini and Wanzira, 2016). In this review, several mechanisms of the action of zinc on acute diarrhea were addressed including its influence on more than 300 enzymes in the body; its promotion of immunity and resistance of mucosal layers and skin to infection, and development of the nervous system. Zinc is also important for antioxidant activity and it preserves thereby the cellular membrane activity. Within the gut, Zn restores mucosal barrier integrity and enterocyte brush-border enzyme activity, and upon intestinal pathogenic burden, it can promote the production of antibodies and circulating lymphocytes, as well as having a direct effect on ion channels, where it functions as a potassium channel blocker of adenosine 3-5-cyclic monophosphate-mediated chlorine secretion. Among studies included in this systematic review (Lazzerini and Wanzira, 2016), the most frequent Zn dose was 20 mg/day, and various types of Zn salts were used. The most frequently used medical zinc source for treating diarrhea in pigs is the ZnO. The Committee for Veterinary Medicinal Products (CVMP) of the European Medicines Agency (EMA) announced in December 2016 that in their opinion they found the risk/benefit balance was unfavourable for the therapeutic use of zinc oxide and that all products containing zinc oxide should have their marketing authorisations removed. However, as concluded by Zentek (2019), it can be assumed that in particular pharmacological zinc concentration in the feed and the subsequent significantly increased zinc concentrations in the digesta are causal for the occurrence of antimicrobial resistances.

The key difference between Zn and ZnO is that Zn is a chemical element (a transition metal), while ZnO is a chemical compound. One may ask why the ZnO (rather than other zinc sources) became the therapeutic source of zinc for treating diarrhea in pigs, when this is not a common Zn source for treating diarrhea in human. This can probably be ascribed to the first nutritional study using dietary ZnO for the prevention and treatment of PWD (Poulsen, 1995), and since its publication, supply of ZnO has received considerable attention, however, the exact modes of action remains to be elucidated. When addressing zinc replacement strategies it is important to note, that Zn can be applied at low dosages (around 150 ppm) to weaners as a nutritional component, while usage in high levels as zinc oxide (around 2,500 ppm) has been shown to be an effective strategy to prevent and control e.g. post-weaning diarrhoea problems (Poulsen, 2019). Hence, in the following, we will address potential dietary strategies for prevention of post diarrhea. In accordance with studies on Zn for children, the prevention by which ZnO have been reported to prevent PWD in pigs point towards both physiological and non-physiological mechanisms. A crucial response during the weaning transition is the feed intake of piglets, and enhanced feed intake of piglets provided dietary ZnO supplementation was in fact obtained (Broom et al., 2003). Improved physiological serum Zn status was observed in the study with weaned piglets in which beneficial effects of increased concentrations of ZnO on post-weaning diarrhea and growth of the piglets were obtained in piglets during the first two weeks after weaning (Poulsen, 1995). Other reports have found changes in

some pancreatic enzymes and hormonal status (Hedemann et al., 2006). Furthermore, expression of genes and proteins related to energy and amino acids metabolism and in parallel accumulation of Zn in liver and pancreas in piglets post weaning after feeding high dietary ZnO for 4 weeks (Pieper et al., 2012), however, this may also lead to increased oxidative stress in these organs. Zn also has major influence on the innate and adaptive immune system (Bonaventura et al., 2015), as shown in pigs where dietary zinc level influenced immune responses of the weaned pig (Kloubert et al., 2018), and Zn enhances intestinal epithelial barrier function (Shao et al., 2017).

The non-physiological mechanisms by which ZnO have been reported to prevent PWD points towards its influence on the gastrointestinal microbiota. Reduced bacterial activity in the digesta from the gastrointestinal tract of pigs fed 2,500 mg/kg as ZnO compared with that in animals receiving 100 mg/kg ZnO, most likely reflecting a reduced load of bacteria present in the gastrointestinal tract of animals receiving the high ZnO level (Højberg et al., 2005). Overall, the research on pigs indicate a major influence of ZnO dose on the gastrointestinal tract microbiota and show that, besides a potential promotion of feed intake, high dietary ZnO doses may further render more energy available for the host animal by generally suppressing the commensal gut microbiota. This has actually also been suggested as one of the working mechanisms behind the effect of antibiotic growth promoters (Collier et al., 2003).

With all these reported mechanisms by which zinc may prevent diarrhea in human and pigs, it is of course difficult to imagine a single food component or feed additive, which can replace zinc for its capability to prevent diarrhea. However, there are nutritional components and bioactive substances, which share some or even several of the modes of action by which Zn is suggested to prevent diarrhea.

Dietary strategies to prevent PWD

There are a number of potential dietary strategies, which have been proposed for preventing PWD, and some are in fact all-ready implemented in practice. Reduction of the dietary protein level is considered an effective strategy to prevent PWD. Since medicinal levels of ZnO have direct antimicrobial effects, potentially targeting specific pathogens such as *E. coli* strains, but also reduce the microbial load in general, and further exerts effects on host-immunity and epithelial barrier functions, such mechanisms are central to prevent progression of ETEC-infection of pigs. These will therefore also be keys to consider in order to identify Zn replacement strategies.

Restriction of protein level

There is a considerable body of research dating back to the 1950s and 1960s which has implicated an association between protein level and diarrhoea after weaning (Pluske, 2013), and as also stated in their review (Kil and Stein, 2010), crude protein is the most important nutrient associated with digestive disorders in the pig. Upon consumption of a meal, pH in the stomach is increased whereby proliferating of *E. coli* is facilitated. Feed ingredients such as soya bean meal, fishmeal and milk powder, which are typical sources of protein used in feed for weaned piglets, have a high buffering capacity, which also can increase stomach pH and thereby limit pepsin activity. Hence, excessive crude protein intake by weaned pigs can lead to increased microbial fermentation of undigested protein, and this is a contributing factor to development of PWD. One of the implemented strategies in practice to limit diarrhoea incidence is the use of low-protein diets, i.e. containing less than 18% crude protein. This strategy is not without challenges because reduction

of the concentration of crude protein for weaned pigs may influence the growth performance because some of the indispensable amino acids may be present in concentrations below the requirement for maximum growth. However, the loss in growth of piglets on low-protein diets in comparison with piglets on diets with a normal protein content may be compensated on the longer term, as the lifetime performance in some studies did not differentiate (e.g. Wellock et al., 2009). Furthermore, crystalline amino acids (lysine, methionine, threonine, tryptophan, isoleucine and valine) in low-protein diets can be used to maintain the balance of the required amino acids for the weanling pigs and thereby pig performance (e.g. (Lordelo et al., 2008)), however, little is known regarding the requirement of specific amino acids during an infectious challenge, when the host immune responses are activated.

Bioactive feed components and feed additives - and their mode of action

As stated above, the various possible modes of action by which a given dietary strategy; a feed additive, or a bioactive feed component may prevent progression of ETEC-infection of pigs, are related to the gut microbiota and/or to the host physiology. Thus, targeted strategies for prevention of ETEC-infection can be ascribed according to the certain step of the pathogenesis as described below, and examples of feed additives and bioactive components playing the mode of action is given in brackets:

- 1) *Protecting the gut from ETEC adhesion and colonization* by reducing the sensitivity of fimbrial receptors on the porcine enterocytes and/or blocking the fimbriae of ETEC (e.g. specific immunoglobulins).
- 2) *Inhibiting the growth of ETEC in the gut* due to bactericidal or bacteriostatic effects (e.g., organic acids, medium-chain fatty acids, fermented liquid feed, antimicrobial peptides, bacteriophages, lysozymes).
- 3) *Maintaining a balanced intestinal microbiota* (e.g., probiotics, prebiotics).
- 4) *Improving the host immune functions* (systemically and locally) including prevention of excessive inflammation (e.g., immunoglobulins, fatty acids, vitamins, trace minerals).
- 5) *Preventing disruption of intestinal mucosal integrity* and/or improving the morphology of the small intestinal epithelium (e.g., specific egg yolk antibodies, bacteriophages).

In the following, the mechanisms by which some of the above-mentioned examples of feed additives considered capable of promoting gut health of pigs and thereby prevent ETEC infection are described (Lauridsen et al., 2019):

One of the main parameters influencing bacterial growth is pH, and therefore reducing the luminal pH, i.e. by addition of *organic acids* or other acidifiers, has an antibacterial effect (Canibe et al., 2005). Lactic acid bacteria are able to grow at relatively low pH, and are therefore more resistant to organic acids than, for example, enterobacteria. Among organic acids, butyric acid has been in focus, especially in human but also in pig research, due to the multiple ways by which this compound affect the host. *Fermented liquid feed (FLF)* is another feeding strategy to reduce the luminal pH (of the stomach). FLF is prepared by mixing water or another liquid, e.g., whey, with

feed and thereafter incubation of this mixture (for a certain period of time, at a certain temperature). As fermentation progresses, lactic acid bacteria proliferate, resulting in high concentration of especially lactic acid and low pH, leading to reduced numbers of coliform bacteria (Canibe and Jensen, 2012). Thereby a reduced load of *E. coli* reaches the ileum, protecting proliferation of pathogens, and maintaining a stable bacterial community.

Antimicrobial lipids such as fatty acids and monoglycerides are promising antibacterial agents that destabilize bacterial cell membranes, causing a wide range of direct and indirect inhibitory effects. Besides, *medium-chained fatty acids* (MCFA) are an immediate energy source for the host and its immune cells, and improve intestinal integrity during inflammatory conditions. Combining MCFA with organic acids are some of the most recent initiatives to identify alternatives to antibiotics (Ferrara et al., 2017), however, more in vivo studies are needed to document the efficacy of these feed additives for preventing PWD as well as solving the challenges of reduced palatability of some MCFA (Lauridsen, 2020). In this context, encapsulation can be used to allow the organic acids (including short-chain fatty acids) to reach the distal small intestine without being absorbed and thereby exert their antibacterial effect at the site of interest. Mono- and diglycerides are produced upon enzymatic hydrolysis of dietary triglycerides, and research has suggested that these fatty acids and monoglycerides exert antibacterial effects against infectious pathogens (Zentek et al., 2011). Using these agents as therapeutics requires improvement of the delivery in order to be applied in vivo.

Bacteriophages are bacteria-targeting viruses, common in all natural environments and very specific as each type generally attacks specific bacterial species. Several studies have evaluated the antimicrobial ability of phages targeting *E. coli*, including the fimbriae types F4 and F18, most commonly associated with piglet PWD. The use of phages is still limited in controlling feed borne pathogens in pigs, and more knowledge is needed to understand essential challenges, including phage resistance, phage-host interactions as well as unwanted perturbations of the gut microbiota.

Probiotics are, by definition, live microorganisms that, when administered in adequate amounts, confer health benefits to the host, and are one of the functional foods that link diet and health. Prebiotics (primarily carbohydrates that resist digestion in the ileum) are defined as selectively fermentable components inducing specific changes in composition and/or activity of the gastrointestinal microbiota and conferring host well-being and health benefits. During the past years, many studies on pre- and probiotics (or their combination also known as synbiotics) have been carried out in pigs. These studies have shown a broad range of beneficial effects in terms of pathogen inhibition and its consequences, including immunological development and fortification of intestinal barrier functions. Because *prebiotics* are readily available substrates for probiotics, prebiotics may improve the survival of concurrently administered probiotic strains. Studies have shown potential capacity of probiotics in terms of immunomodulatory activities, but contrasting effects can also be obtained, which is probably due to differences with respect to the probiotic strain used, experimental settings, diets, initial microbiota colonization, administration route, time and frequency of administration of the probiotic strain and sampling for analysis (Roselli et al., 2017).

Passive immunization, i.e. the administration of *antibodies* (immunoglobulins) for protecting the host against infections, is not a new idea and seems to constitute a real and widely applicable

alternative to antibiotics in modern animal production (Hedegaard and Heegaard, 2016). Oral administration of specific chicken IgY has been shown to be effective against a variety of intestinal pathogens, including ETEC (Diraviyam et al., 2014). Use of blood plasma or purified porcine immunoglobulin G from pooled natural pig plasma is also of interest as an immune-enhancing technology. Yeast derivatives based on *Saccharomyces cerevisiae*, where the bioactive components are mannans and β -glucans, may be immunomodulatory and can prevent colonization of pathogenic *E. coli*. However, verification is needed on the exact composition and dosage of the bioactive components of the yeast derivatives, as well as use of antibodies, specifically when to apply and dose required.

Other active ingredients such as *vitamins, and trace elements* and *antimicrobial peptides* (AMPs), have a major influence on the immune system. As recently reviewed (Lauridsen et al. 2021), vitamins have been much less considered in relation to gut health and function of pigs although they share a lot of similar functions with Zn, and several recent studies in relation to human intestinal health and disease have been published. Once the immune system is activated, the nutrient partitioning is altered and partly directed to produce immune molecules and inflammatory responses. Reduction of inflammatory responses (for instance by acetylsalicylic acid and vitamin E) and oxidative stress reactions (by using antioxidative enzymes and vitamins) during the activation of the immune cells may protect against severity of infectious disease, including disruption of epithelial barrier function and mucosal injury, and eventual subsequent septic shock due to toxins entering the system. Thus, the support on the immune system via vitamins and trace elements (selenium, copper, and zinc) is important, and any transient deficiency during the weaning due to low feed intake or malnutrition should be avoided. Furthermore, some vitamins, trace elements, clay, and carbohydrates etc. exert specific actions on intestinal barrier integrity by influencing (size and/or activity) of goblet cells of the ileum and hence protecting the epithelial barrier function.

During recent years, blends or combinations of the above-mentioned feed additives have been investigated in relation to gut health effects in pigs, which seems a reasonable strategy as one molecule cannot prevent e.g. progression of *E. coli* infection at all steps. In this context, algae, plant components (e.g. dried plant material), plant extracts and essential oils, berries and fruit extracts have gained interest in terms of potential benefits on gut health in pigs. These 'food'-related components may encompass a natural cocktail of antibacterial and immunomodulatory agents. However, the proposed bioactivity of the given cocktail, and its stability in feeds as well as sensory acceptance should be investigated further. Likewise, dairy-based products gained increasing interest as a potential nutritional tool to prevent piglets from developing diarrhoea due to ETEC (Sugiharto et al., 2015). Bovine colostrum, milk and milk fractions such as whey and casein contain several bioactive components with antimicrobial and immunomodulatory properties, but the knowledge related to the application of the dairy-based products to prevent ETEC infection and post-weaning diarrhoea is very limited.

Conclusion and perspectives

Although the mechanisms behind the success of high dietary ZnO levels in terms of limiting PWD in pigs are still not clear, they are considered to be related both to an impact on the gut microbiota

and to the physiological effects of zinc on the animal. Protein restriction is crucial for prevention of PWD, and in addition, there is however, there is probably not a single feed additive, which can replace ZnO for its capability to prevent PWD. However, many potential feed additives share several of the mechanisms by which Zn may influence the ETEC infection and development of diarrhea. The ideal feed additive for enhancing gut health of pigs is probably a cocktail of various antibacterial and immunomodulatory agents targeting the specific challenges of the microbiome-host interaction as *E. coli* infection progresses in piglets post-weaning.

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Identification and Validation of Biomarkers for Designing Enteric Methane Mitigation Strategies in Cattle

Identification et validation de biomarqueurs pour la conception de stratégies d'atténuation du méthane entérique chez le bovin

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Abstract

This paper provides an update of research on enteric methanogenesis by reporting data from recent case studies on promising methane (CH₄) mitigation strategies and biomarkers to estimate CH₄ emissions in cattle. We demonstrated an additive and persistent effect between lipids and nitrate for reducing rumen methanogenesis in cows. This proof of concept opens up a range of possibilities for designing new strategies to increase CH₄ abatement. Despite a high individual variability in daily CH₄ emissions among animals, the dairy cow ranking was not stable over time across the different diets. Our data highlight the importance of phenotyping animals across environments in which they will be expected to perform. In growing bulls, enteric CH₄ methane emissions are positively associated to residual feed intake in growing bulls suggesting that animals that ingested food in excess of their maintenance and growth requirements emitted more CH₄ per day with both diets. A meta-analyse approach indicated that milk fatty acids have better potential to accurately predict CH₄ emissions when combined with other variables (e.g, days in milk, diet composition) compared with on their own. Inhibition of enteric methanogenesis in dairy cows induced changes in plasma metabolome highlighting metabolomic shifts and potential new markers of CH₄ emissions.

Résumé

Cet article présente une mise à jour de la recherche sur la méthanogénèse entérique et rapporte les données d'études de cas récentes portant sur des stratégies de réduction du méthane (CH₄) et des biomarqueurs prometteurs qui pourraient permettre d'évaluer les émissions de CH₄ chez les bovins. Nous avons démontré un effet additif et persistant entre les lipides et le nitrate pour réduire la méthanogénèse dans le rumen des vaches. Cette preuve de concept ouvre un éventail de possibilités pour la conception de nouvelles stratégies visant à obtenir des réductions de CH₄ accrues. Malgré une grande variabilité des émissions quotidiennes de CH₄ entre les individus, le classement des vaches laitières n'était pas stable dans le temps avec les différents régimes alimentaires. Nos données soulignent l'importance du phénotypage des animaux dans les environnements dans lesquels ils seront amenés à produire. Chez les taureaux en croissance, les émissions de CH₄ entérique sont positivement associées à la consommation d'aliments résiduels, ce qui suggère que les animaux qui ingèrent un excès d'aliments par rapport à leurs besoins d'entretien et de croissance émettent plus de CH₄ par jour avec les deux rations. L'approche

basée sur une méta-analyse montre que les acides gras du lait permettraient de prévoir avec plus de précision les émissions de CH₄ lorsqu'ils sont combinés à d'autres variables (p. ex., jours de production, composition de la ration) que si on les considère seuls. L'inhibition de la méthanogénèse entérique chez les vaches laitières a induit des changements dans le métabolome plasmatique, ce qui permet d'identifier des changements métabolomiques et de nouveaux marqueurs potentiels des émissions de CH₄.

Introduction

The major challenge in livestock farming worldwide is to increase and improve production while limiting environmental impact and competition for resources for human consumption. Ruminants are key to food security as they convert forages, not directly usable by monogastric animals and humans, into animal products for human food. They supply 51% of all protein with respectively 67% and 33% from milk and meat (Gerber et al., 2013). However, ruminant livestock contributes substantially to greenhouse gas (GHG) emissions (14.5% of total anthropogenic emissions). Cattle (beef, milk) are responsible for about two-thirds of that total, largely due to methane (CH₄) emissions resulting from rumen fermentation, and with a global warming potential 28 times that of carbon dioxide (IPCC, 2014).

Developing operational strategies to reduce enteric CH₄ emissions in cattle is important, while maintaining optimal production to preserve the competitiveness of farms, and meeting consumer demand for safe and quality food. Many strategies (nutrition, biotechnologies, management and genetics) have been evaluated individually worldwide, but few cost-effective solutions are currently available to producers (see review by Beauchemin et al., 2020). The future challenge in significantly and sustainably mitigating methanogenesis may be to design new strategies, such as combining feeding management and animal genotyping for low CH₄ emissions.

Another important issue is the quantification of CH₄ emissions to evaluate the effectiveness of different mitigating strategies. Under controlled conditions available in experimental research stations, direct measurement methods are commonly implemented (Guyader et al., 2015; Arbre et al., 2016; Doreau et al., 2018). These methods are accurate and repeatable, but are not adapted to a large number of animals and are difficult to implement on farms. To address these issues, alternative methods of estimating CH₄ emissions using biomarkers (or proxies) are currently under evaluation (see review by Negussie et al., 2017).

The purpose of this paper is to provide an update of research on methanogenesis by reporting data from recent case studies on promising CH₄ mitigation strategies and large-scale proxies to estimate CH₄ emissions in cattle.

Combination of Strategies to Mitigate Enteric Methane Emissions

Numerous CH₄ mitigation strategies have been proposed in recent decades (Martin et al., 2010). Most concern manipulation of the rumen microbiome by nutrition (lipids, starch, natural and chemical additives, etc.) or by biotechnologies (vaccines, probiotics, early life programming, etc.). Strategies focusing on the animals (genetic, etc.) are more recent and have been reviewed

by Beauchemin et al. (2020), who considered not only their potential of CH₄ mitigation, but also their expected availability and feasibility of implementation on farms, as well as their limits. It is important not to focus on a single strategy of CH₄ mitigation, but to investigate several at the same time for diverse production systems and environments. The future challenge in significantly and sustainably mitigating enteric CH₄ emissions in ruminants may be to combine different strategies, such as feeding management and animal genotyping for low CH₄ emissions (Beauchemin et al., 2020).

Additive Effect of Different Anti-methanogenic Dietary Strategies

Lipids have emerged as a persistent option for mitigating enteric CH₄ emissions from ruminants. However, their potential of mitigation is moderate (~20%) if used at a suitable dose, while avoiding negative effects on animal performance (Martin et al., 2021). We tested whether it was possible to increase the CH₄ mitigation potential of lipids (linseed oil) by combining them with another dietary strategy (nitrate) with a different mode of action on the metabolism of H₂ in the rumen. In a meta-analysis, we reported that lipids may be relevant in reducing H₂ production (via reduction of protozoa), whereas nitrate may stimulate H₂ consumption (H₂ sink) by a pathway of competition with methanogenesis (Guyader et al., 2014). We assumed that simultaneous manipulation of H₂ production and H₂ utilization allows a greater reduction in CH₄ emissions than when acting on a single pathway. To test this hypothesis, we investigated the effect of linseed oil and nitrate distributed alone or in combination on CH₄ emissions and digestive processes in non-lactating cows fed a hay-based diet (Guyader et al., 2015). The persistency of the effect of linseed+nitrate on CH₄ emissions and lactating performance was also assessed in dairy cows fed a maize silage-based diet (Guyader et al., 2016).

Compared with the control diet, linseed oil and nitrate decreased CH₄ emissions [g/kg dry matter intake (DMI)] by -17 and -22% when fed alone, respectively, and by -32% when combined, without altering diet digestibility. The daily kinetics of CH₄ emission measurements in respiration chambers clearly showed the mode of action of each dietary treatment. Linseed oil supplementation reduced CH₄ emissions throughout the day compared to the control diet, while nitrate had a transient but marked action for 3 h post-feeding. Combination of the strategies cumulated the two modes of action (**Figure 1**).

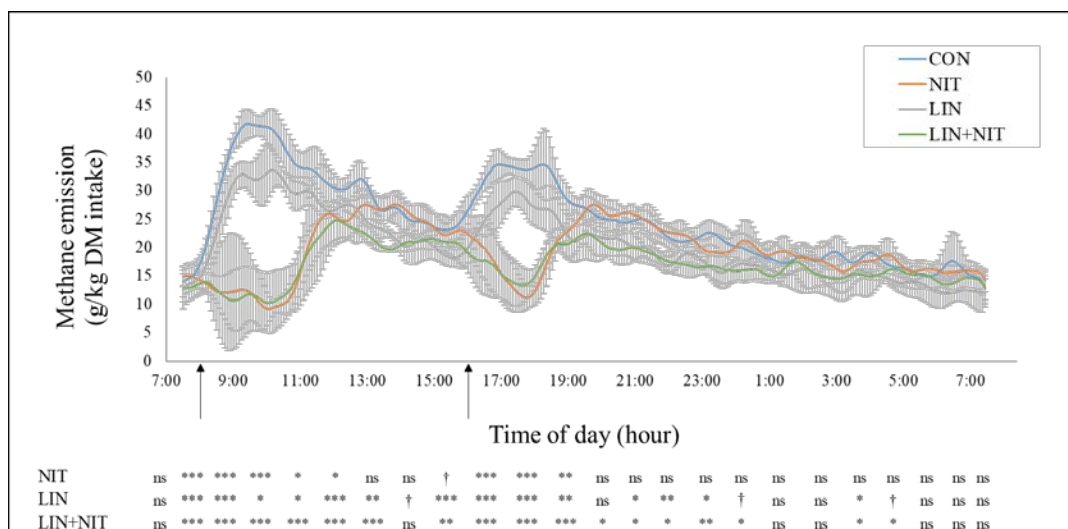


Figure 1. Daily methane production pattern of non-lactating cows fed 4 different diets containing linseed oil and calcium nitrate alone or in combination (n = 4). Treatments consisted of control diet (CON), CON plus 3% calcium nitrate (NIT), CON plus 4% linseed oil (LIN) and CON plus 4% linseed oil and 3% calcium nitrate (LIN+NIT). The arrows indicate time of feeding. Error bars indicate SD. Symbols indicate hourly statistical comparison between dietary treatments ($\dagger P \leq 0.10$; $*P \leq 0.05$; $**P \leq 0.01$; $***P \leq 0.001$). Adapted from Guyader et al. (2015).

In addition, we showed that linseed oil + nitrate fed to lactating cows for 2 months induced a repeatable and persistent reduction of CH₄ emissions [−47%, g/d; −30%, g/kg DMI; −33%, g/kg fat- and protein- corrected milk (FPCM)], on average; **Figure 2**), without any effect on digestibility of nutrients, nitrogen balance or animal health. Intake and milk yield tended to be lower for dairy cows fed the linseed oil + nitrate diet, but feed efficiency (kg FPCM/kg DMI) was unaffected. Nitrate or nitrite residuals were not detected in milk and associated products (yoghurts, whey, curd and 6-week ripened Saint-Nectaire cheese) suggesting that milk from cows fed nitrate is safe for human consumption. This persistent effect showed the absence of adaptation of the rumen microbiota. However, the energy benefits from the decreased CH₄ emissions with the linseed oil + nitrate diet did not appear beneficial for dairy performances.

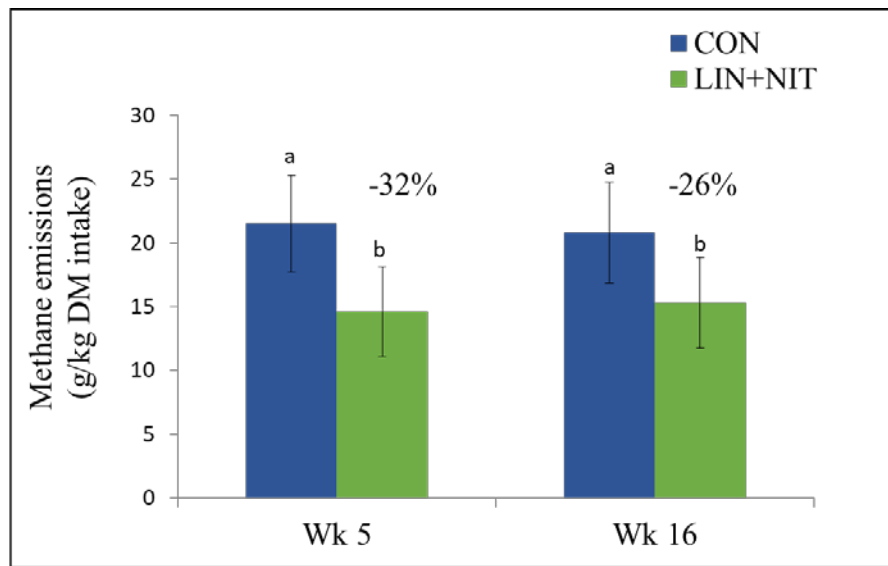


Figure 2. Methane emissions from lactating cows fed a control diet based on maize silage (CON; n = 8) or CON supplemented with 10% extruded linseed plus 1.8% nitrate on a DM basis (LIN+NIT; n = 8) after 5 weeks (Wk 5) and 16 weeks (Wk 16). Error bars indicate SD. Letters indicate a significant effect ($P = 0.002$) between diets. Adapted from Guyader et al. (2016).

This work confirmed our initial hypothesis that combining dietary strategies with different mechanisms of action to reduce H₂ availability in the rumen reduces methanogenesis more markedly than when lipids and nitrate are fed individually. This proof of concept opens up a range of possibilities for designing new strategies to increase CH₄ abatement.

Animal Phenotyping for Low Methane Emissions across Diets

Animal phenotyping as a strategy to mitigate enteric CH₄ emissions is more recent and less known than strategies related to nutrition (review of Løvendahl et al., 2018). Interest in selecting ruminants for their CH₄ emissions has increased since it was demonstrated that heritability of this trait is moderate ($h^2 \approx 0.29$ and 0.40 for CH₄ in g/d in sheep and cattle, respectively) (Pickering et al., 2015; Lassen and Løvendahl, 2016). Together with the heritability of traits, classification of individuals by their CH₄ emissions and stability of ranking over time for different diets are prerequisites for the purposes of animal breeding.

The repeatability of CH₄ emissions in a large number of animals in contrasting feeding conditions is a key point to consider. Coppa et al. (2020) investigated under farm conditions the repeatability [i.e. between animals/(between animals + unexplained) variability] and stability of dairy cow ranking in the long term according to their CH₄ emissions. Forty-five dairy cows fed three contrasted diets formulated to be more or less methanogenic were phenotyped over a 4-month period after the peak of lactation regarding their CH₄ emissions quantified using GreenFeed systems. The repeatability of CH₄ emissions was calculated from data averaged over 1, 2, 4, and 8 weeks for each animal. It increased with the duration of the measurement period and peaked (0.78 for CH₄ in g/d) with 8-week averaged periods. This high repeatability confirmed a high variability among cows, which is relevant for phenotyping regarding CH₄ emissions. However, despite this high individual variability, the dairy cow ranking on CH₄ emissions (g/d) was not stable over time between all individuals or within any of the three diets (**Figure 3**). Similar results were found by Rischewski et al. (2017) who showed an unstable ranking in a few dairy cows ($n = 8$) fed a similar diet between three periods (early, middle and late) in the second phase of lactation. These authors stated that only the 2 extreme cows (the highest and the lowest CH₄ emitters) stayed approximately (but not absolutely) near the extreme, whatever the lactation phase of measurement. Coppa et al. (2020) found that, in a large number of animals, even extreme cows changed ranking. Denninger et al. (2019) showed that differences in daily CH₄ emissions (g/d) between 2 groups of 10 high- or low-emitter dairy cows were maintained across 5 subsequent months of lactation and 2 different diets, but no information on individual ranking independently of group was available.

Excluding reasons related to the methodological analysis, the most probable interpretation of the ranking variation over time is a change in CH₄ emissions within individuals. Dietary factors (DM and GE intakes) or milk performances (milk yield and FPCM) are known to explain variability of absolute CH₄ emissions over time in dairy cattle (Beauchemin et al., 2020). However, in our trial, ranking stability was not improved when expressing CH₄ emissions per unit of intake or unit of milk, irrespective of the diets (**Figure 3**). Other factors like changes in animal physiological status over time (i.e. pregnancy, heat events, etc.) or external factors like climatic changes (i.e. occurrence of heat stress period) or barn management activities (i.e. insemination practices, etc.) could perturb feeding behavior over time (i.e. variations in meal frequency and consistency) and thus CH₄ emissions (Garnsworthy et al., 2012; Hammond et al., 2016). Our data highlight the importance of phenotyping animals across environments in which they will be expected to perform.

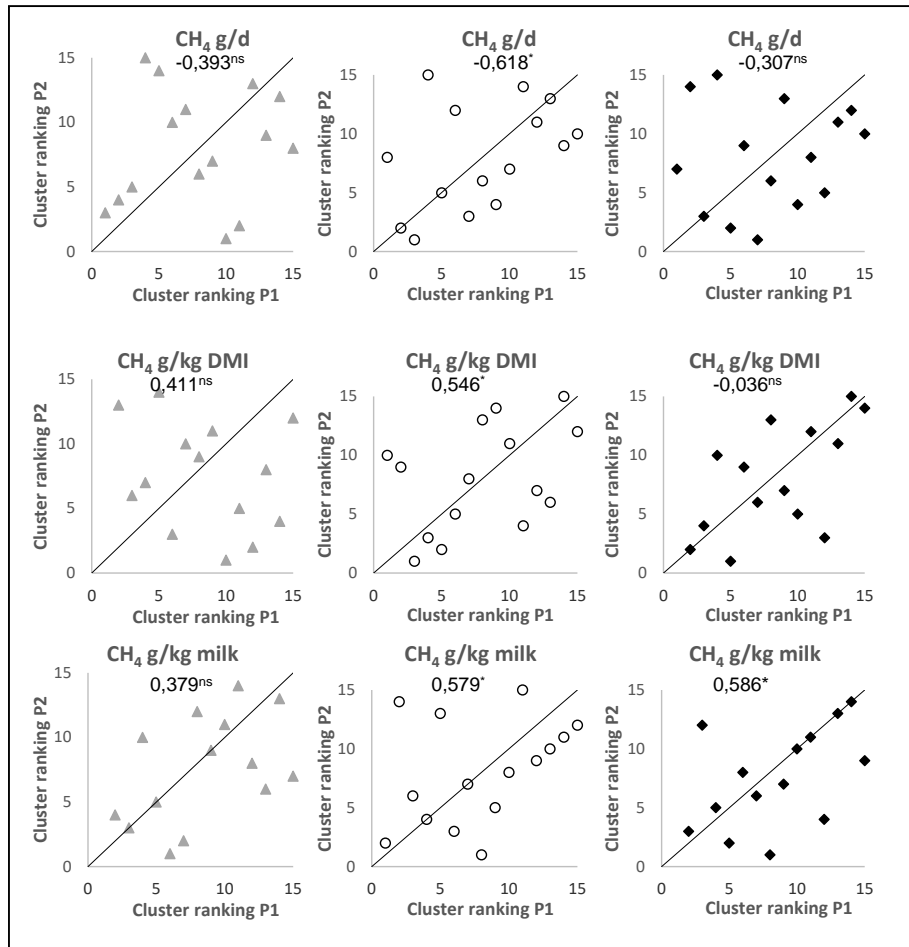


Figure 3. Individual ranking of CH₄ emissions from 2 subsequent periods of 8 weeks (P1: week 11–18 and P2: week 19–26) for diet CH₄⁺ (grey triangles; n = 15), CH₄^{int} (white dots; n = 15), and CH₄⁻ (black squares; n = 15) diets; full lines represent the y = x equations; (Rs = Spearman correlation coefficients between the ranking of the same cow in the two subsequent measurement periods); *, P < 0.05; ns, not significant. CH₄⁺, diet formulated to produce high methane emissions; CH₄^{int}, diet formulated to produce intermediate methane emissions; CH₄⁻, diet formulated to produce low methane emissions. Adapted from Coppa et al. (2020).

The profitability of ruminant livestock is assumed to increase if their environmental impact is reduced through the selection of low CH₄ emitters. Indeed, the feed efficiency of animals would increase by saving energy lost in the form of CH₄, and CH₄ emissions expressed per kg of product would decrease (Capper et al., 2009; Legesse et al., 2016). The relationship between the two phenotypes (CH₄ emissions and feed efficiency) is largely unknown (Basarab et al., 2013) due to the absence of simultaneous measurements on these two large-scale criteria over sufficiently long and representative breeding periods, and is also difficult to investigate because of the complexity of their mode of expression.

Bes et al. (2021) recently investigated the relationship between the individual variability of enteric CH₄ emissions to that of feed efficiency in growing steers fed two contrasted diets. One hundred young Charolais bulls fattened for 6 months received ad libitum a total mixed ration

based either on maize silage (rich in starch; MS-S) or on grass silage (rich in fiber; GS-F). Methane emissions (g/d) were measured individually using GreenFeed systems and individual feed efficiency was calculated as residual feed intake (RFI = difference between the real and theoretical amount of DM intake in kg/d) during the same periods.

The RFI values ranged between -0.98 and 1.08 (SD = 0.59) for the MS-S diet and between -0.93 and 0.82 (SD = 0.42) for the GS-F diet. Methane emissions differed between the two diets [between 201 and 371 g/d (SD = 31) and between 154 and 329 g/d (SD = 29) for MS-S and GS-F diets, respectively; $P < 0.01$]. Regression analysis revealed that daily CH_4 emissions (g/d) were positively linked with RFI ($r = 0.50$, $P \leq 0.01$) with a slope averaging 26 g/kg DM intake for both diets (**Figure 4**). Animals that ingested food in excess of their maintenance and growth requirements emitted more CH_4 per day with both diets. These data are in agreement with those reported recently in dairy heifers (Flay et al., 2019) and steers when CH_4 emissions were expressed in g/kg DMI (Renand et al., 2019). In contrast, two older studies showed that CH_4 emissions (g/d) were lower for the more efficient steers (Hegarty et al., 2007; Nkrumah et al., 2006).

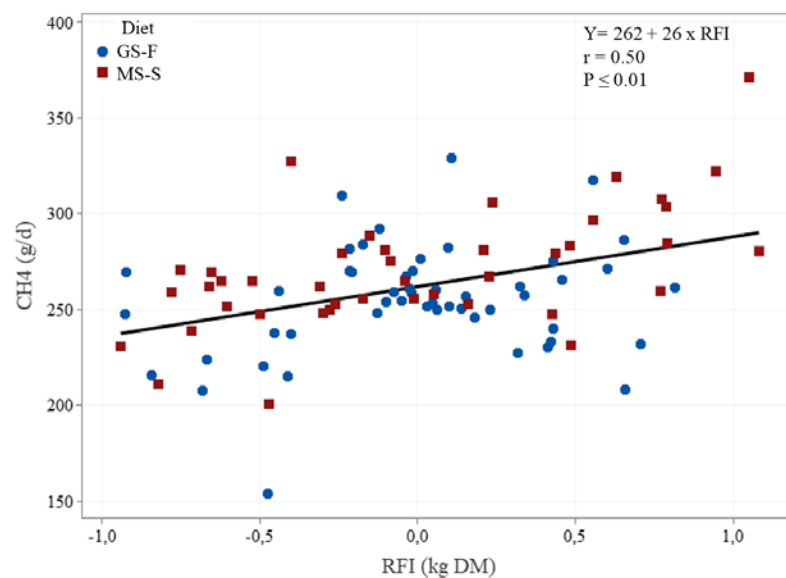


Figure 4. Relationship between individual CH_4 emissions and RFI in growing bulls ($n = 100$) fed a diet based on maize silage (rich in starch; MS-S) or based on grass silage (rich in fiber; GS-F). Adapted from Bes et al. (2021).

All these results confirm the complexity of studying the relationship between these 2 criteria and the need for further research in order to understand the underlying mechanisms. Incorporating CH_4 production in a genetic selection program needs to consider the potential risks of counter-selecting other phenotypes of interest (Løvendahl et al., 2018). In their review, these authors report that the genetic selection of animals that emit low CH_4 would reduce the digestive efficiency of fiber, a phenotype which is particularly interesting to consider in the context of ruminant breeding that does not compete with human food production.

Biomarkers (or proxies) to Estimate Enteric Methane Emissions

In the last few years, much progress has been made in terms of the precision and accuracy of direct methods of measuring daily enteric CH₄ emissions (g/d). However, most methods used to quantify these emissions on an individual scale in ruminants (respiration chambers, SF₆ tracer technique, GreenFeed®) remain expensive, labor-intensive, technically challenging and thus limited to research (Hammond et al., 2016). There is a need to develop easy methods of estimating CH₄ emissions on a large scale and in practical conditions, so as to evaluate the effect of different mitigating strategies (diets, animals, management systems) compared to what is currently done in controlled experimental conditions.

Negussie et al. (2017) reviewed a large range of available CH₄ proxies that have been recently explored in dairy cattle, including parameters related to (1) intake, (2) digestive function, (3) milk performance, (4) the whole animal. All proxies were summarized in terms of their characteristics, including simplicity, cost, accuracy, invasiveness, and throughput.

In this section, biomarkers (or proxies) are defined as ‘indicators’ measurable in body matrices easy to access, and that can be used to predict CH₄ emissions. Methane proxies might be less accurate than direct methods, but can be measured frequently to reduce random noise. We focused on proxies from two body matrices: milk routinely obtained on dairy farms, and plasma for its potential availability in all cattle including beef and growing replacement dairy heifers.

Proxies from milk

Proxies related to milk composition, like milk fatty acid (MFA) and mid-infrared (MIR) profiles, seems to be a promising way to predict CH₄ emissions because precursors for methanogenesis and de novo synthesis of MFA both arise in the rumen (Negussie et al, 2017). Milk FA composition was first used as CH₄ emission proxies in dairy cows fed corn silage-based diets containing linseed (Chilliard et al., 2009). Other prediction equations based on milk FA concentrations have been reviewed by van Gastelen and Dijkstra (2016), but all were developed in a narrow range of diets and had limited data numbers, thus restricting their range of application.

In this context, Bougouin et al. (2019) used a meta-analytical approach to construct a set of CH₄ prediction equations from MFA in lactating dairy cows fed a wide range of diets. A large international data set¹ (n = 825) was created including individual CH₄ emissions (mostly measured using a respiration chamber and SF₆ tracer techniques), individual MFA profile (exclusively determined by gas chromatography), and data on daily DMI, dietary composition, milk performances (yield and composition), and animal characteristics (days in milk, body weight).

Twenty equations for prediction of enteric CH₄ have been published. Equations including MFA alone confirmed common rumen metabolic pathways between methanogenesis and lipid metabolism in dairy cows. **Figure 5** presents the most correlated individual MFA among the 5

¹With 312 observations from published and unpublished experiments (17 experiments) by INRAE-UMRH (France), 119 individual observations (5 experiments) from Aarhus University (Denmark), 218 observations (7 experiments) from Ellinbank Research Centre (Australia), and 177 observations (5 experiments) from AAFC Sherbrooke (Canada).

main families (SFA, odd- and branched-chain FA, cis MUFA, trans MUFA, and PUFA) with 3 CH₄ emission metrics (g/d, g/kg DMI, g/kg milk). Prediction equations based on MFA alone had a root mean squared error of 65.1 g/d, 2.8 g/kg of DMI, and 2.9 g/kg of milk, respectively. Complex equations that additionally used other variables [DMI, dietary chemical composition (neutral detergent fiber, ether extract contents), animal characteristics (days in milk, body weight)] had a lower root mean squared error of 46.6 g/d, 2.6 g/kg of DMI, and 2.7 g/kg of milk, respectively.

Few MFA (cis-11 C18:1) and (trans-10 C18:1) were commonly found among prediction equations in this study and in the literature (Dijkstra et al., 2011; Mohammed et al., 2011; Rico et al., 2016; van Gastelen et al., 2018). Performance of the prediction equations was not consistent, meaning that MFA used alone had a limited potential to predict CH₄ emissions. Our results confirmed that equations predicting CH₄ from MFA depend on diet (Mohammed et al., 2011; Dijkstra et al., 2016; Rico et al., 2016) and the lactation stage (Vanrobays et al., 2016). In addition, increasing the complexity of prediction equations, by combining proxies, increased their robustness (Negussie et al., 2017), probably because more complex equations explain an additional proportion of the variability not taken into account in simple equations with MFA alone.

A minimum difference of 16% (simple equations) and 11% (complex equations) in CH₄ emissions (g/d) between mitigating strategies can be evidenced with the best prediction equations (Bougouin et al., 2019). These authors highlight the low potential of applicability of their equation on farms, because of the limitations (time-consuming, expensive) associated with routine use of gas chromatography, which is considered the gold standard in determining MFA.

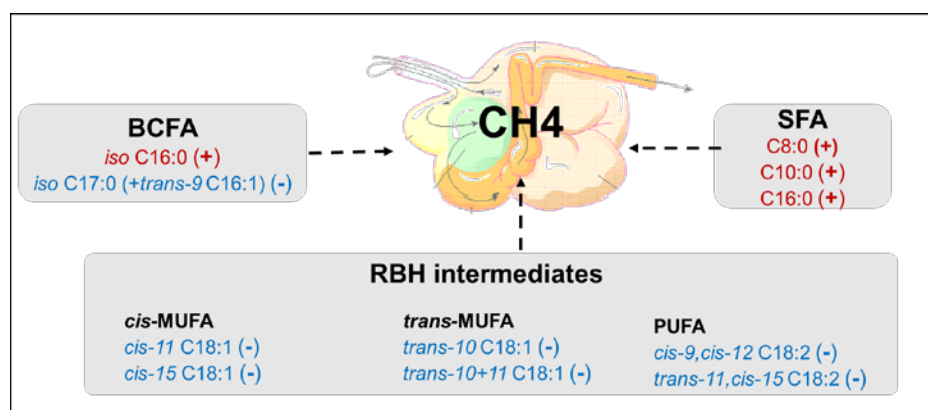


Figure 5. Significant correlation between CH₄ emissions (g/d, g/kg DMI, g/kg milk) and individual MFA in dairy cows. BCFA = odd- and branched-chain FA; SFA = saturated FA; RBH = rumen biohydrogenation intermediates with MUFA = mono-unsaturated FA and PUFA = polyunsaturated FA. (+) and (-) indicate positive and negative correlation, respectively. Adapted from Bougouin et al. (2019)

Milk composition (fat, protein, lactose and urea contents), including some FA (Soyeurt et al., 2011), can be determined routinely and at low cost by MIR spectroscopy in milk recording laboratories. Some authors report a good potential of milk MIR spectra as a proxy for prediction

of CH₄ emissions in dairy cattle, especially when combined with animal characteristics such as lactation stage (van Gastelen and Dijkstra, 2016), milk yield, parity and breed (Vanlierde et al., 2020). This high throughput approach allows CH₄ production to be incorporated in dairy cow breeding programs.

Proxies from plasma

A plasma metabolomics approach was successfully used to assess performance efficiency in growing steers (Artegoitia et al., 2017) and heat stress in dairy cows (Tian et al., 2015). Information is scarce on the metabolic consequences in the host ruminant when rumen methanogenesis is depressed. Understanding these possible changes is important for acceptance by producers of mitigation strategies, and for exploring new metabolites that could be used as proxies of enteric CH₄ emissions. In addition, plasma is a biological fluid that has the advantage of being easily accessible from all types of ruminants.

Yanibada et al. (2020) hypothesized that plasma metabolites that originate from rumen microbes, from the host or both (co-metabolites), are impacted by a reduction of ruminal methanogenesis. Twenty-five Holstein primiparous cows were fed the same diet with (n=12) or without (n=13) a specific inhibitor of methanogenesis for 6 weeks to obtain two groups of cows classified as low- or high- CH₄ emitters according to their different (23%) CH₄ emissions (g/d). Milk production, food consumption, body weight and indicators of the health status of animals were comparable between all animals. The plasma metabolome was explored using untargeted [nuclear magnetic resonance (NMR) and liquid chromatography-mass spectrometry (LC-MS)] and targeted (LC-MS/MS) approaches.

The plasma metabolome differed between high- and low-CH₄ emitter dairy cows, although the differences were moderate. A wide range of discriminating plasma metabolites (n=48) were identified and 7 metabolomic pathways were associated in the low-CH₄ emitters (Figure 6). Some metabolites were of microbial origin, such as dimethyl sulfone, formic acid and metabolites containing methyl groups such as stachydrine. They are known to be involved in methanogenesis or the use of H₂ in the rumen and can potentially be used as proxies of methanogenesis. Other discriminating plasma metabolites are produced by the host or are of mixed microbial-host origin. The latter metabolites, which increased in low-CH₄ emitters, belong to general energy and amino acid metabolic pathways, suggesting that reduction of methanogenesis occurs without negative effects on dairy cows.

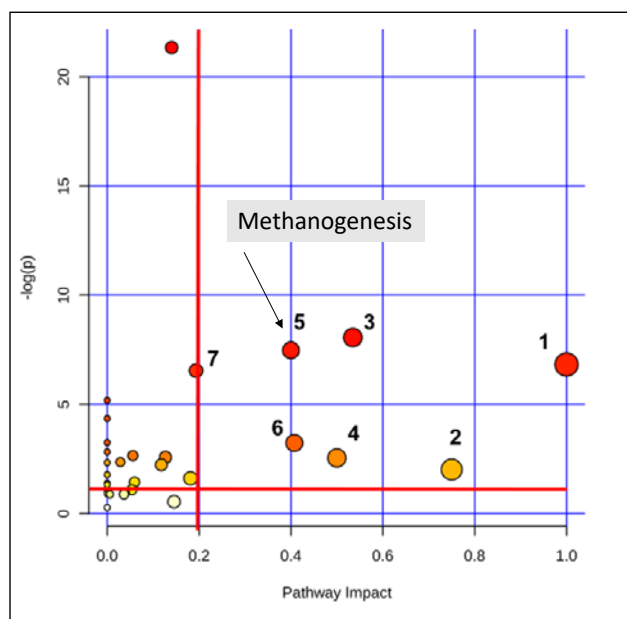


Figure 6. Analysis of the metabolic pathways modified in low-CH₄ emitters. Discriminating metabolites identified showed 7 impacted metabolic pathways: **1)** Valine, leucine and isoleucine biosynthesis **2)** Taurine and hypotaurine metabolism **3)** Glycine, serine and threonine metabolism **4)** Phenylalanine, tyrosine and tryptophan biosynthesis **5)** Methane metabolism **6)** Glyoxylate and dicarboxylate metabolism **7)** Arginine and proline metabolism. The plot was built based on the pathway enrichment analysis (node colors) and on the pathway impact values resulting from the pathway topology analysis (node size). Adapted from Yanibada et al. (2020)

Yanibada et al. (2020) demonstrated the proof of principle that plasma metabolome reflects changes in enteric CH₄ emissions in dairy cattle. Plasma metabolites identified were useful in improving understanding of the physiological effects on the ruminant host induced by a reduction in methanogenesis. Plasma is a biofluid of interest for developing new proxies of methanogenesis. Validation of metabolomic approach and discriminant metabolites is required in studies using anti-methanogenic additives with different modes of action, in a large number of animals and in different production conditions.

In conclusion, large-scale studies covering a wide range of experimental conditions is necessary to increase the accuracy of the existing prediction equations and to validate new proxies. The integration of databases from different matrices and reconciliation of all the data would offer the possibility of proposing combinations of even more robust and discriminating models for prediction of individual emissions of enteric CH₄ in ruminants. The development of proxies is at a relatively early stage and should be a priority in future research for designing new enteric methane mitigation strategies in cattle.

Acknowledgements

The author thanks the organizing committee of the 5th annual Animal Nutrition Conference of Canada (ANCC) for their invitation and encouragement to prepare this article. The author thanks the technical and scientific staff, permanent and non-permanent, of the UMR 1213 Herbivores for their input in this collaborative research as well as institutes and private partners for their financial support [Adisseo France SAS (Antony, France), Agrial Caen, France), APIS-GENE (Paris, France), Deltavit (Janze, France), DSM Nutritional Products AG (Kaiseraugst, Switzerland), Institut de l'Élevage (Paris, France), Lallemand (Blagnac, France), Moy Park Beef Orleans (Fleury-les-Aubrais, France), ADM nutrition animale - Neovia (Saint-Nolff, France), Techna France Nutrition (Coueron, France), and Valorex (Combourtille, France)].

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Abstract

Until now, individual measurements of feed efficiency and feeding behavior have been performed on animals raised alone and in cages, but it has several drawbacks, in particular the absence of social interactions and the limitation of movements, which leads to an experimental bias, as conditions are not representative of farming conditions. The electronic feeder BIRD-e (Bird Individual Ration Dispenser-electronic) developed by INRAE and ITAVI allows to overcome these constraints with automatic recording of individual measurements of feed consumption and poultry weight raised on the ground and in groups. BIRD-e has eight feed access points, each equipped with two scales (one for feed, one for birds) and a detection antenna. Each chick is fitted with an electronic identification chip so that each visit to the feeder is recorded. Each station can record data from 100 to 120 animals raised on the ground, without human intervention and without penalizing their growth. Therefore, individualized measurements of weight and feed consumption are obtained continuously. It is also possible to collect information on feeding behavior: time spent to eat, time between two meals, etc. The expected benefits of this technology are at first progress in genetic selection, by integrating criteria of feeding behavior and by being more efficient on feed efficiency. On the nutritional aspect, this innovative tool allows to study the impacts of feed composition and technology on the feeding behavior of birds and their performance. This device is already used in research programs and could be used for partnership research within a year.

Résumé

Jusqu'à présent, les mesures individuelles de l'efficacité alimentaire et du comportement alimentaire ont été effectuées sur des animaux élevés seuls et en cage; cette méthode présente toutefois plusieurs inconvénients, notamment l'absence d'interactions sociales et la limitation des mouvements, ce qui conduit à un biais expérimental, car les conditions ne sont pas représentatives des conditions d'élevage. La mangeoire électronique BIRD-e (Bird Individual Ration Dispenser-electronic) développée par l'INRAE et l'ITAVI permet de surmonter ces contraintes avec l'enregistrement automatique des mesures individuelles de consommation d'aliments et de poids des volailles élevées au sol et en groupe. BIRD-e dispose de huit points d'accès aux aliments, chacun équipé de deux balances (une pour les aliments, une pour les oiseaux) et d'une antenne de détection. Chaque poussin est équipé d'une puce électronique d'identification afin que chaque visite à la mangeoire soit enregistrée. Chaque station peut enregistrer les données de 100 à 120 individus élevés au sol, sans intervention humaine et sans pénaliser la croissance des animaux.

Ainsi, des mesures individualisées du poids et de la consommation d'aliments sont obtenues en continu. Il est également possible de recueillir de l'information sur les habitudes alimentaires : temps passé à manger, temps entre deux repas, etc. Les bénéfices attendus de cette technologie sont, dans un premier temps, des progrès dans la sélection génétique par une meilleure intégration des critères de comportement alimentaire et d'efficacité alimentaire. Sur l'aspect nutritionnel, cet outil innovant permet d'étudier les impacts de la composition des aliments et de la technologie sur le comportement alimentaire des oiseaux et sur leurs performances. Ce dispositif est déjà utilisé dans des programmes de recherche et pourrait être utilisé pour des travaux réalisés en partenariat d'ici un an.

Introduction

Controlling production costs and animal welfare are important factors for breeders. Feed accounts for 50 to 70% of the production cost in poultry production (van Horne, 2018). The feed conversion ratio (FCR), which is the ratio of feed consumption to meat or egg production, is an important criterion for the profitability of these farms. It is also an indicator of the environmental impact of production. For example, the reduction of 10% in FCR results in a reduction in the production cost of live chicken of about 6%, but also a reduction in environmental impact of 12% for nitrogen excretion and 17 % for phosphorus (De Verdal et al., 2011). So far, individual measurements of feed efficiency and feeding behavior have been carried out on animals reared in individual cages, but this method shows several drawbacks, including lack of social interactions with other animals and movements limitations, which leads to experimental bias, as conditions are not representative of actual production conditions. The bias is particularly important in the case of alternative productions such as slow-growing lines, which have more physical activity. In addition, there is a need of individual data for geneticists to make progress in genetic selection, by being more accurate and comprehensive on feed efficiency and having the possibility to integrate data relating to feed intake dynamics. This technology could enable the study of the impacts of nutritional and technological characteristics of feed on birds feeding behavior and performances. Access to daily performances would make the study of critical time point such as feed transitions easier. In addition, access to individual data would make it possible to obtain greater statistical power from tests with a much smaller number of animals. This is why researchs were initiated in 2005 by INRAE to develop a tool to measure individual broilers feed consumption of broilers, leading to a patent registration (Chabault et al., 2013). Since then, various improvements have been made to the feeder, such as automatic data recording and processing, better ergonomics and constructions materials, and the addition of systems for animal weighing (Guettier et al., 2020; Mika et al., 2021). Therefore, the purpose of this paper will be to briefly describe the electronic dispenser and its use, and to give an example of application.

Description of the electronic feed station

BIRD-e has a circular shape with adjustable feet for leveling and stability of the automaton. A cylindrical PVC outer shell protects the internal elements. The inner unit is vertically movable thanks to an electric column fixed to the base, in order to adapt the feeder height to the animals' size.

The feeder is equipped with eight independent feed accesses, without corridors, so that the chickens can express their natural feeding behavior. Each access includes one feed tube, one feed trough, one antenna placed on the top of the feed trough to detect the animal's RFID chip, one scale for recording feed weight, and one scale for recording animal weight placed under the tray on which the animal would climb to eat (Figure 1). One feed station is suitable for 100 to 120 animals without causing competition.

Feed is distributed through eight independent columns (1 column for each access). Each column has its own load cell. The measuring capacity of the load cells for the feed is 10 Kg. Each column has a storage volume of 5.6 dm³ and can fill about 3 Kg of feed. The lower part of the feed columns is the feed access for the animals. To avoid waste, the feed access is covered with a transparent cover through which the animal passes its head to eat.

Animals are weighed using interchangeable trays according to their growth: they are clipped onto the animal scale in the axis of the eight accesses. Each tray has its own scale. The measuring capacity of the weighting cells for the animal is 30 Kg.

The RFID antennas are fixed on the feeder, at the entrance of each access. They are adjustable in height, and in the axial position by means of the sliding support and its knurled knob. This system allows the antenna to be positioned as close as possible to the animal's chip throughout the rearing period, which is particularly useful when animals are young.

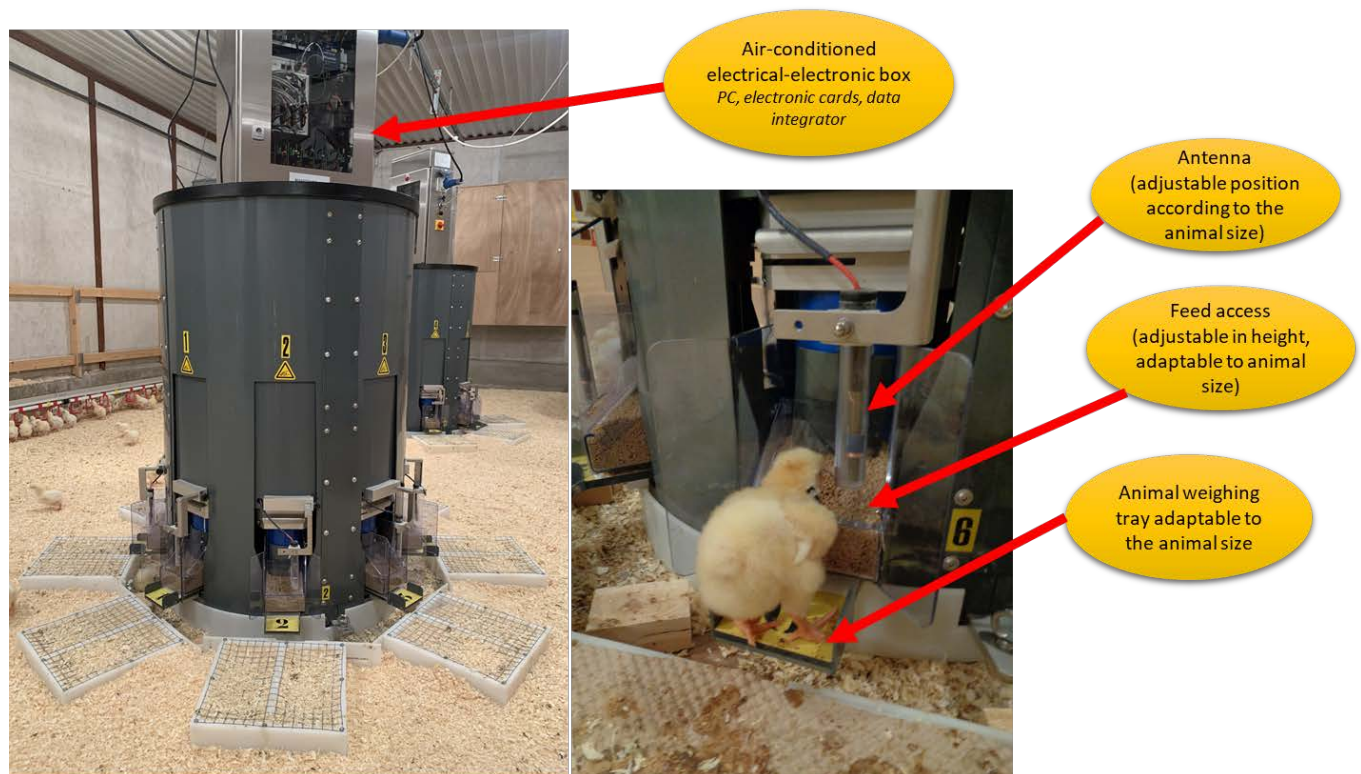


Figure 1. Description of BIRD-e, the electronic feeder for poultry

Operation of the electronic feeder and data collected

At one day of age, animals are equipped with a RFID (Radio Frequency Identification) electronic chip attached to the base of the neck for individual identification (Figure 2).

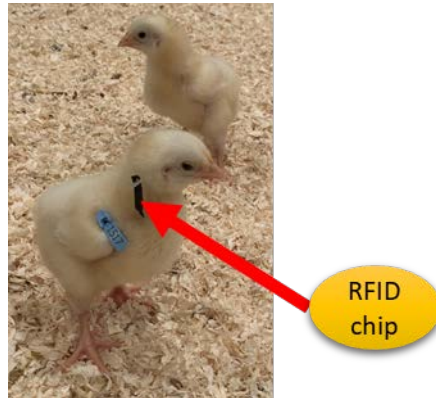


Figure 2. RFID chip attached to the base of the neck for individual identification.

When the animal comes to eat, it climbs onto the tray and the chip is identified by the electronic sensor. Sensor is connected to an electronic card that transmits all the identification data to a data acquisition system (PMX, HBM), and then stored in an industrial computer. All computer and electronic equipment are grouped together in the air-conditioned electrical cabinet located above the feeder.

Raw data obtained from the station were 1) feed weight by access every second, 2) animal identity, time, and access number every time an antenna detected a chip, and 3) average animal weight at each visit. A visit was defined by consecutive readings of the same chip at the same access with less than 10 seconds between consecutive chip detections. All scales and antennas were connected to a central data acquisition system.

The identification data is stored in a first file with the following elements: "Date - Time - Feeder number - Access number - Identification number". The animal weight is recorded every time an animal chip is detected. A second data file is generated and contains, for each visit of the animal to the feeder, the "Date - Number of the feeder - Access number – Start time of the visit – End time of the visit - Average weight of the animal during the visit ". The feed weight in each of the feed columns is recorded in real time, second by second, whether an animal is present or not, thanks to the scales attached to each feed column. The resulting file contains one data item per second and per access and the following information is entered: "Date - Time - Feeder number - Access number - Feed weight".

The data acquisition system synchronizes the data from the eight accesses, then transmits it to the central computer, which stores it and then exports it to a server. An algorithm for calculating feed consumption for each visit was developed by determining feed access and access weight at the start and the end of the visit. It allows to determine, for a defined feed access, the total amount of feed consumed per animal. By adding up all the daily feed accesses of a given animal, it is then possible to have access to the amount of feed ingested each day.

In addition to animal performance data (weight, feed intake, feed efficiency), the BIRD-e feeder provides access to new data such as animal feeding behavior. Then, it is possible to know, for each animal, the number of feed accesses made in a day, the time interval between two feed accesses, the average duration of a feed access, the feed intake intensity, etc. This opens up new perspectives for future feeding trials.

Example of application: Study of the interaction between genotype and diet for consumption, feeding behavior and feed conversion ratio (Berger et al., 2021)

In this study, we evaluate the adaptation ability of two genotypes, i.e. fast (FG) and slow-growing (SG) chicken, with different levels of growth rates and nutritional requirements, to a diet containing a mixture of alternative feedstuffs. Birds from both genotypes were reared separately, at different periods. In the first batch, 80 SG male chickens were reared from 1 to 82 days, according to “Label Rouge” rearing practices. In the second batch, 80 FG male chickens were reared from 1 to 35 days. Two different diets were designed, adapted to each genotype nutritional needs: a classic corn-soybean diet as a control (Control) and an alternative diet (Alternative) including more wheat and local rich-protein feedstuffs as sunflower meal and rapeseed meal, and less soybean meal than the control diet. Within a genotype, diets were isoproteic and isoenergetic. At one day of age, animals were identified with a RFID chip for individual identification and were randomly distributed in two pens, each pen receiving either the control or the alternative diet. In each pen, the 40 animals have access to four accesses of the feeder. Daily body weight and feed intake of each animal were continuously recorded throughout the experiment thanks to the BIRD-e feeder, as well as feed intake dynamics. The kinetics of mean body weight (BW), feed intake (FI), feed efficiency and feed intake dynamics, as well as the variability of these traits between the alternative diet and the control diet were compared from hatch to slaughter.

Within each genotype, BW, FI and FCR of animals receiving either the Control diet or the Alternative diet were very similar, showing the good capacity of animals of both genotypes to adapt to the alternative diet (Figures 3 and 4). We can also see on these figures that, above the mean values, this feed station allows to calculate the coefficient of variation (CV) of each trait, it is thus also possible to check the adaptation to the new feed through the observation of impact on the homogeneity of the group, and not only on the mean value. The CV for BW in both genotypes was stable and low at all ages, usually lower than 20% (Figure 3).

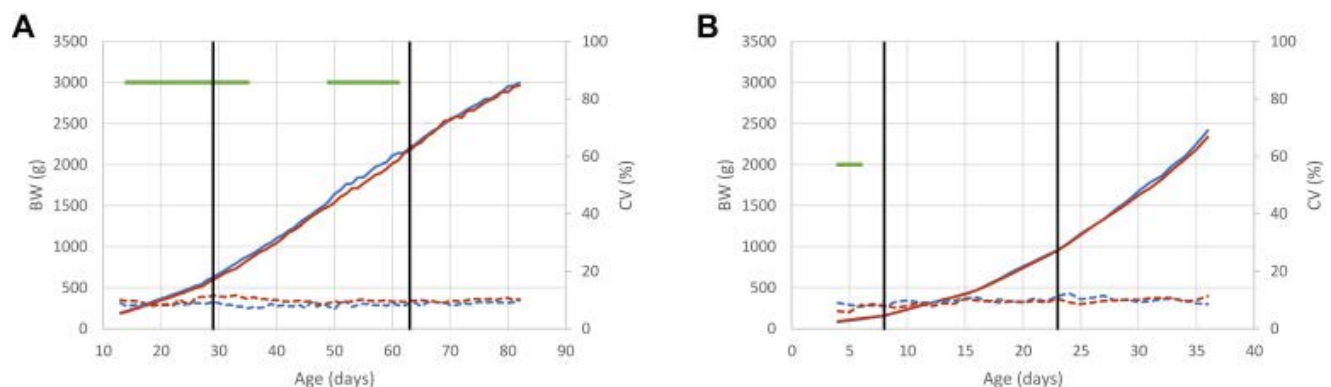


Figure 3. Body weight of animals from SG (A) and FG (B) chickens receiving a control (red) or alternative (blue) diet. Dotted lines represent coefficient of variation of data. Vertical lines represent diet changes. Horizontal green bars indicate significant differences between the two diets. Reproduced from Berger et al. (2021).

However, change with age of daily FI and daily FCR coefficients of variation differed between traits and genotypes, although similar trends were found between diets. The general trend was an increase in the CV of the two traits with age in SG chickens (Figures 2A, 2C) and a decrease in FG chickens (Figures 2B, 2D). Differences of variability between diets for daily FI and daily FCR were strong in FG line. Alternative diet led to a decrease in the variability of those performances during the grower and finisher phases in FG chickens. In the case of SG chickens, the CV differed between diets during these phases for daily FI. When significant, performances were less variable with the Alternative diet than with the Control diet.

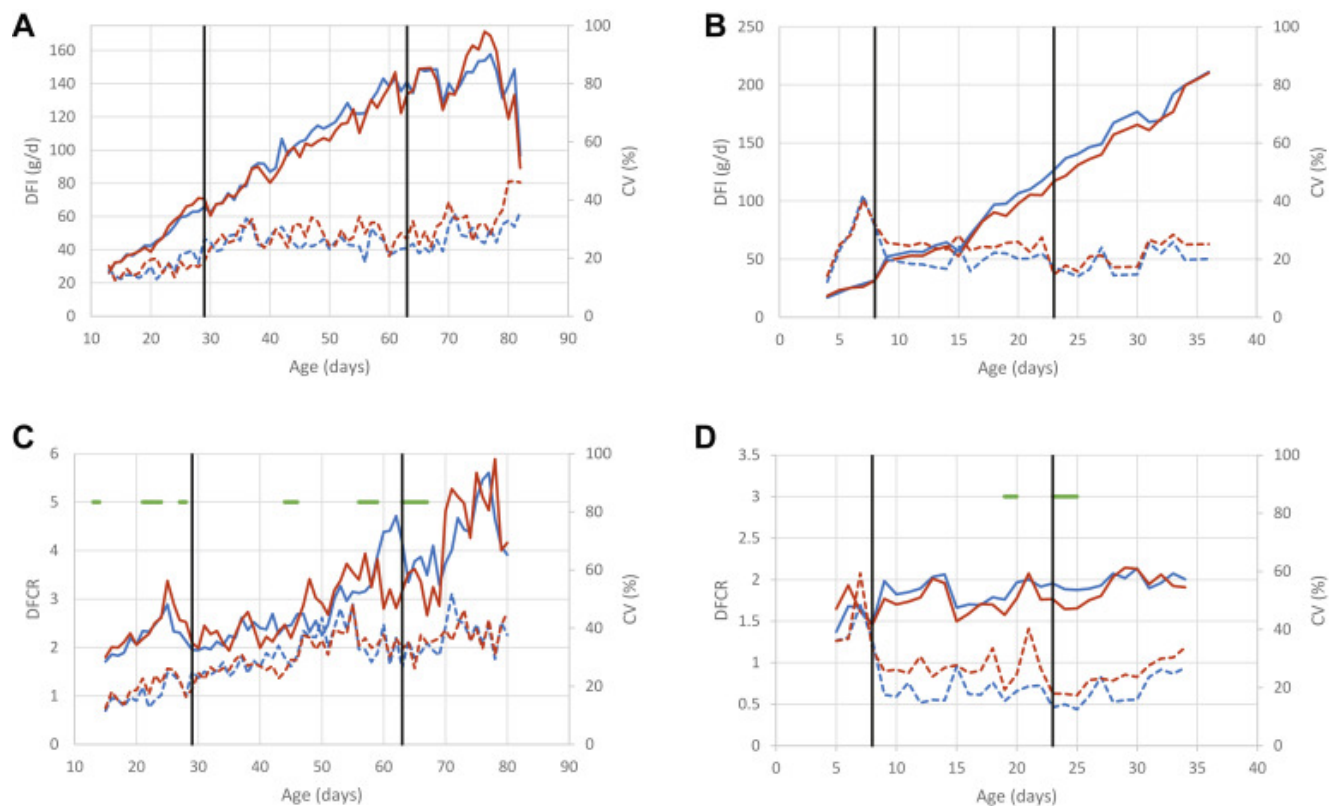


Figure 4. Feed intake and daily feed conversion ratio from SG (A, C) and FG (B, D) chickens receiving a control (red) or alternative (blue) diet. Dotted lines represent coefficient of variation of data. Vertical lines represent diet changes. Horizontal green bars indicate significant differences between the two diets. Reproduced from Berger et al. (2021).

Regarding feed intake dynamics, SG and FG chickens have on average the same number of feed accesses per day except for FG animals fed the control diet, where higher feed access was observed (Figure 5). However, a significant difference in the average feed access duration was observed between the two genotypes, and the two diets only for FG chickens. FG animals have significantly longer feed access than SG animals, which is consistent with their higher feed intake. In the SG

line, no difference was observed on this criterion between the diets. However, in the FG line, animals with alternative diet have a longer feed access than animals with the control diet. It can be assumed that this difference between the two diets is due to a different bulkiness, which in turn is due to a higher level of fibers in the alternative diet.

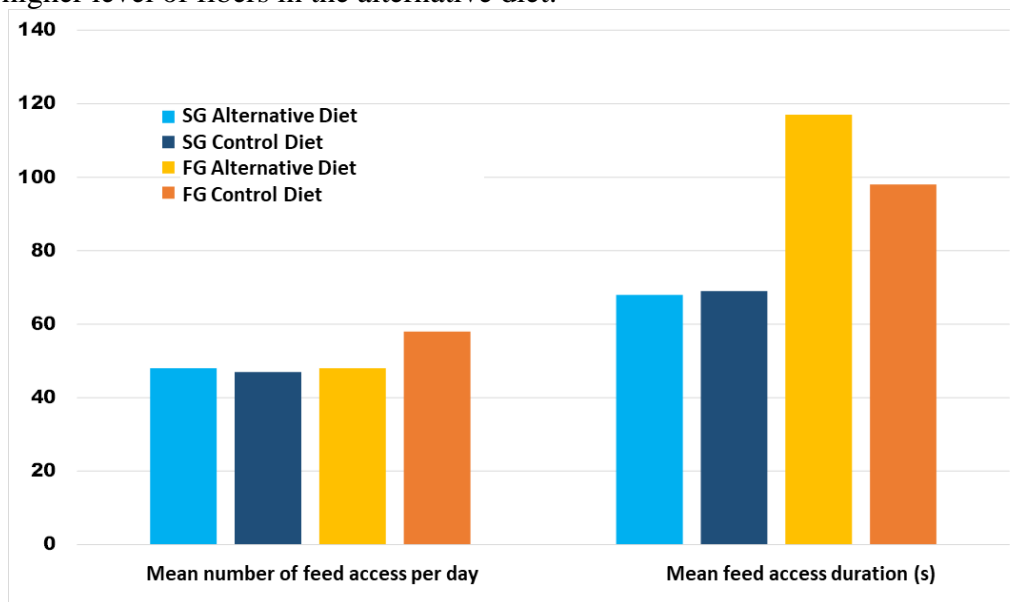


Figure 5. Feed access number and duration for animals from two genotypes and receiving two different diets.

The current application study showed that differences between the two diets are moderate in terms of final performances in both genotypes, indicating that chickens are able to adapt to a diet composed of a mixture of alternative feedstuffs. The most striking difference in the adaptability of chickens to the Alternative diet was found in the variability of performances. Animals fed with Alternative diet had more homogeneous performances for FI and daily FCR, especially in FG chickens. To go further, this design has recently been used to determine genetic parameters of daily data of feed intake and feed efficiency in broilers.

Conclusion

With the BIRD-e feed station, it is possible to conduct experiments under production conditions (on the floor and in groups) and to collect individual measurements of feed intake, body weight and feed intake dynamics. The application example presented here illustrates the interest of this device with the study of different genetics and feeds.

The application study highlighted other interesting aspects of BIRD-e. For example, this device allows us to closely examine consumption during diet changes with a short-term reaction (in relation to sensory changes in diet) and a medium-term reaction (in relation to metabolic effects). The BIRD-e automatic consumption machine represents a real innovation for research and offers new perspectives around genetic selection and animal nutrition. This automaton allows experimentation without altering animal's behavior and well-being. The impact of this technology is an expected progress in genetic selection, by integrating feeding behavior criteria and being

more efficient on feed efficiency. This innovative tool makes it possible to study the impacts of feed composition and technology on the birds feeding behavior and performance. Today, six electronic feed stations have been developed and used by INRAE and ITAVI for their own research programs. It could be used for a research partnership within a year.

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Basic Elements, Difficulties and Pitfalls on the Development and Application of Precision Nutrition Techniques for Smart Pig Farming

Concepts de base, difficultés et pièges lors du développement et l'application de techniques de nutrition de précision pour les élevages de porc intelligents

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Abstract

Smart farming (SF) is an emerging concept that refers to managing farms using modern information and communication technologies where interconnected smart sensors and devices, big data analysis, and conventional and artificial intelligence models will provide a new edge for innovation and productivity. SF is part of the precision livestock farming (PLF) concept that by automatically and continuously monitoring and controlling farm processes will help farmers to improve management tasks such as monitoring of animal performance and health, and optimization of feeding strategies. An important component of SF and PLF is precision livestock nutrition (PLN). PLN consists in providing in real time to individuals or group of animals the amount of nutrients that maximizes nutrient utilization and farm performance. The use of PLN in growing-finishing pig operations can decrease protein intake by 25%, nitrogen excretion into the environment by 40%, while increasing profitability by nearly 10%. The success of the development of SF and PLN depends on the automatic and continuous collection of data, data processing and interpretation, and the control of farm processes. The advancement of PLN requires the development of new nutritional concepts and mathematical models able to estimate individual animal nutrient requirements in real time. Further advances for these technologies will require the coordination of different experts (e.g., nutritionists, researchers, engineers, technology suppliers, economists, farmers, and consumers) and stakeholders. For the adoption of SF and PLN the development of integrated user-friendly systems and the end-user training is imperative. The development of PLN will not just be a question of technology, but a successful marriage between knowledge and technology in which improved and intelligent mathematical models will be essential components.

Résumé

L'agriculture intelligente est un concept émergent qui fait référence à la gestion des exploitations agricoles à l'aide des technologies modernes de l'information et de la communication, où les capteurs et dispositifs intelligents interconnectés, l'analyse des mégadonnées et les modèles d'intelligence conventionnelle et artificielle procureront un nouvel avantage en matière d'innovation et de productivité. L'agriculture intelligente fait partie du concept d'élevage de précision qui fait appel à la surveillance et au contrôle automatiques et continuels des processus agricoles pour aider les producteurs à améliorer les tâches de gestion, telles que le suivi des performances et de la santé des animaux et l'optimisation des stratégies d'alimentation. Un élément important des concepts d'agriculture intelligente et d'élevage de précision est la nutrition de précision. La nutrition de précision consiste à fournir en temps réel à des individus ou à un groupe d'animaux une quantité de nutriments permettant de maximiser l'utilisation des éléments nutritifs et les performances de l'exploitation. La nutrition de précision dans les élevages de porcs en croissance-finition peut réduire la consommation de protéines de 25 % et l'excrétion d'azote dans l'environnement de 40 %, tout en augmentant la rentabilité de près de 10 %. Le succès de l'agriculture intelligente et de la nutrition de précision repose sur la collecte automatique et continue de données, sur le traitement et l'interprétation des données et sur le contrôle des procédés d'exploitation. Le perfectionnement de la nutrition de précision passe par le développement de nouveaux concepts nutritionnels et modèles mathématiques capables d'estimer les besoins nutritifs individuels des animaux en temps réel. Les progrès futurs de ces technologies nécessiteront la coordination de différents experts (par exemple, spécialistes de la nutrition animale, chercheurs, ingénieurs, fournisseurs de technologies, économistes, producteurs agricoles et consommateurs) et autres intervenants. Pour favoriser l'adoption de l'agriculture intelligente et de la nutrition de précision, il est impératif de développer des systèmes intégrés conviviaux et de former les utilisateurs finaux. Le développement de la nutrition de précision ne reposera pas que sur la technologie, mais aussi sur un mariage réussi entre connaissances et technologie et qui comportera obligatoirement des modèles mathématiques améliorés et intelligents.



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Maximizing the Feed Value of High Fibre Forages to Ruminants: The Impact of Ammonia Fibre Expansion Technology (AFEX) and Novel Recombinant Fibrolytic Enzymes on Digestion and the Rumen Microbial Population

Maximiser la valeur alimentaire des fourrages riches en fibres pour les ruminants : l'impact du procédé d'expansion des fibres à l'ammoniaque (AFEX) et des nouvelles enzymes fibrolytiques recombinantes sur la digestion et la population microbienne du rumen

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Abstract

Ruminants are unique in their ability to convert high fibre forages and crop residues into high quality meat and milk protein for humans. Although the rumen microbiome is often considered the most efficient microbial system at degrading lignocellulosic biomass, often more than 50% of the structural carbohydrates in cereal crop residues are not digested. This low digestibility of crop residues limits their feed value for ruminants. Increasing the efficiency of utilization of fibrous feedstuffs is essential to increase the sustainability of ruminant production systems from both an economic and environmental perspective. This article will focus on two technologies, ammonia fiber expansion (AFEX) and recombinant fibrolytic enzymes that we have recently worked with to improve ruminal fibre digestion of cereal crop residues. Understanding the factors limiting plant cell wall degradation by rumen microbes is essential for developing strategies to improve forage utilization by ruminants. Dietary supplementation with fibrolytic enzymes is not a new concept and they have been shown to improve ruminal fibre digestion. However, results have been inconsistent. Here we discuss the results of our recent work aiming to identify effective recombinant fibrolytic enzymes developed specifically for the rumen environment. We also show that AFEX technology can dramatically improve ruminal fibre digestion, but it still needs to be industrialized. Recombinant enzymes can potentially improve fibre digestion in the future but improvements are likely to be less than that achievable with AFEX.

Résumé

Les ruminants possèdent la faculté unique de convertir les fourrages à haute teneur en fibres et les résidus de cultures en protéines de viande et de lait de haute qualité pour l'homme. Bien que le microbiome du rumen soit souvent considéré comme le système microbien le plus efficace pour dégrader la biomasse lignocellulosique, souvent plus de 50 % des glucides structurels des résidus de cultures céréalières ne sont pas digérés. La faible digestibilité des résidus de cultures limite leur valeur alimentaire pour les ruminants. Il est essentiel d'accroître l'efficacité de l'utilisation des aliments fibreux pour augmenter la viabilité des systèmes de production de ruminants, tant d'un point de vue économique qu'environnemental. Cet article portera principalement sur deux technologies, soit l'expansion des fibres à l'ammoniaque (AFEX) et les enzymes fibrolytiques recombinantes, avec lesquelles nous avons récemment travaillé pour améliorer la digestion des fibres ruminales des résidus de cultures céréalières. Comprendre les facteurs qui limitent la dégradation des parois cellulaires végétales par les microorganismes du rumen est essentiel pour développer des stratégies visant à améliorer l'utilisation du fourrage par les ruminants. La supplémentation des rations avec des enzymes fibrolytiques n'est pas un concept nouveau et ces produits ont fait la preuve qu'ils améliorent la digestion des fibres ruminales. Cependant, les résultats sont inégaux. Nous discutons ici des résultats de nos récents travaux visant à identifier des enzymes fibrolytiques recombinantes efficaces, développées spécifiquement pour l'environnement du rumen. Nous montrons également que la technologie AFEX peut améliorer considérablement la digestion des fibres dans le rumen, quoique les procédés industriels doivent encore être développés. Les enzymes recombinantes pourraient éventuellement améliorer la digestion des fibres, mais les améliorations seraient vraisemblablement inférieures à celles qui pourront être obtenues avec la technologie AFEX.

Introduction

The world population is expected to increase by ~2 billion, reaching 9.7 billion in 2050 (UN, 2019). Compared to 2012, this growth of the global population and affluence of emerging economies is predicted to promote a 40 to 55% increase in global demand for meat and dairy products by 2050 (FAO, 2018). Land resources available to expand production is limited, and there is increasing societal pressure to reduce the degradation of natural resources. In addition, animal production, in particular beef production, has been criticized based on the use of grain that could be used as food for humans, the use of arable land to produce animal feed (instead of producing food for humans directly), and its contribution to greenhouse gas (GHG) emissions (Mottet et al., 2017). Hence, meeting this increase in the demand for livestock products will require more sustainable production practices and efficient utilization of feeds.

Lignocellulosic cereal crop residues, such as straw and corn stover, are low-cost and widely available forage sources; however, their utilization in ruminant diets is limited due to their low digestibility (< 500 g/kg; Beauchemin et al. 2019). Cereal crop residues have intrinsically low feed value because cereal crops are grown mainly focussing on maximizing grain yield, with little consideration for the residual of forage portion of the crop. Cereal crop residues are characterized by having high fibre (> 70% NDF) and lignin (> 7.0% ADL), and low protein (< 6.5% CP) content (Beauchemin et al. 2019), traits associated with its low digestibility, and consequent low feed

intake (Wilson and Mertens, 1995). The cell wall carbohydrates (fibre) are the main component of these cereal crop residues accounting for up to 80% of the organic matter, and the extent of degradation by rumen microorganisms of this fraction is directly related to the amount of energy available for animal production (Jung and Allen, 1995). These carbohydrates, particularly hemicellulose, are associated with a variety of side groups including acetyl, ferulic/coumaric acid and glucuranoic acids, with covalent linkages to lignin. The lignin that links to these carbohydrates is not degraded in the rumen as this process requires oxygen, which is lacking in the rumen. Hence, the lignin/phenolics complexes reduce digestibility by restricting the access of rumen microbes and enzymes to the carbohydrates within plant cell walls.

Development of technologies to maximize the conversion of low quality forages, like cereal crop residues, into energy within the rumen has the potential to improve the overall efficiency of ruminant production systems from both economic and environmental standpoints. This review will focus on two technologies, ammonia fibre expansion (AFEX) and recombinant fibrolytic enzymes, to improve the ruminal fibre digestion of low quality forages. Many studies in the last 60 years have evaluated the ability of alkali treatments (NaOH, NH₃, Ca(OH)₂, KOH, and CaO) to improve ruminal digestion of corn cobs and stalks, and wheat, barley, rice and oat straws (Klopfenstein and Owen, 1981). However, variability in the results, and safety, environmental, technical, and economical concerns have limited the widespread adoption of alkali treatment of forages for ruminants. Similarly, much work has been done evaluating the effect of adding fibrolytic enzymes to ruminants diets with responses found to be inconsistent, an outcome that has also limited the adoption of this technology (McAllister et al., 2001; Beauchemin et al., 2003; Adesogan et al., 2014; Meale et al., 2014; Arriola et al., 2017). Recent investments in these technologies for the production of biofuels from lignocellulose, and advancements in our ability to study the rumen microbiome and express novel recombinant enzymes have created new opportunities for improving the utilization of low quality forages by ruminants.

Ammonia Fibre Expansion Technology (AFEX)

Process

Originally developed for the pre-treatment of biomass for cellulosic ethanol production, AFEX is an ammonia thermochemical pre-treatment of recalcitrant lignocellulosic biomass. The AFEX treatment is a dry-to-dry process where steam and alkaline anhydrous ammonia are applied to the fibrous substrate in a reaction chamber at high pressure (200-400 psi) and temperature (80-150°C) for < 1 h, followed by a rapid pressure release and ammonia recovery by steam stripping (Mor et al., 2018). The potential health and safety hazards associated with ammonia volatilization, that have limited the use of traditional ammoniation on farm, have been greatly improved by the development of AFEX technology as most (~98%) of the ammonia is recovered for re-use (Teymouri et al., 2005; Mor et al., 2018). Similar to other alkali treatments, AFEX treatment of cereal crop residues can promote cleavage of the hydrogen, α -ether and ester bonds between lignin and hemicellulose, and the linkages between hydroxycinnamic acids (i.e., p-coumaric and ferulic acids) and hemicellulose, consequently disrupting the plant cell wall structure (Xiao et al., 2001). By decreasing the crystallinity and promoting the swelling of cellulose, alkali treatments promote the attachment of rumen microbes to plant cell walls, a process which is essential for ruminal fibre degradation. Compared to other alkali treatments, AFEX is a more intensive process (Bals et al.,

2010), that partially solubilizes hemicellulose, enhances the fragility of the fibre, and increases the surface area available for microbial attachment. This has been hypothesized to improve the accessibility of carbohydrate-active enzymes (CAZymes) to the biomass, increasing the rate and extent of ruminal fibre degradation (Beauchemin et al., 2019).

As a result of ammoniation, crude protein concentration of AFEX-treated cereal residues has been shown to increase anywhere from 81 to 312% compared to the untreated forages (Bals et al., 2010; Blümmel et al., 2018; Beauchemin et al., 2019; Saleem et al., 2019; Passetti et al., 2020). The greater CP content with AFEX treated forages can reduce the need for protein supplementation in the diet of ruminants, as this non-protein N can be used to meet the soluble N requirement of the rumen microbes and contribute to microbial protein synthesis (Scott et al., 2011). The solubilisation of hemicellulose by AFEX is evident by the reduction in NDF without any changes in ADF content. Our previous studies have shown that NDF content of AFEX treated crop residues was reduced by 15 to 24% (Beauchemin et al., 2019; Saleem et al., 2019; Passetti et al., 2020).

Rumen Microbial Population

Mammalian herbivores, including ruminants, do not express the cellulolytic or hemi-cellulolytic enzymes that are involved in degrading ingested plant material. Herbivores rely on a complex gut microbiome composed of a diverse range of bacteria, fungi, and protozoa to perform this function. The rumen microbiome is comprised of bacteria (up to 10^{11} cells/mL), protozoa (10^4 - 10^6 cells/mL), fungi (10^3 - 10^6 zoospore/mL), methanogens (10^6 cells/mL) and bacteriophages (10^7 - 10^{10} particles/mL) that have evolved to specialize in degrading lignocellulosic biomass (Morgavi et al., 2013). The ruminant host is dependent on the enzymes produced by this microbial community to breakdown complex fibrous substrates and convert them into fermentable sugars.

When feed enters the rumen it is rapidly colonized by bacteria and fungi (Edwards et al., 2008; Piao et al., 2014; Huws et al., 2016). There have been extensive studies on the process of feed colonization (McAllister et al., 1994; Edwards et al., 2008; Huws et al., 2013; Huws et al., 2016). The currently accepted model of feed colonization was proposed by Huws et al. (2016) and involves a multi-step process. The epiphytic microbes attached to the surface of feed are rapidly displaced by rumen bacteria within the first hour that feed enters the rumen. This leads to the formation of a stable, “primary colonizing” community within the first 4 hours feed is in the rumen. This diverse primary colonizing community metabolizes readily accessible carbohydrates and nutrients in feed (Huws et al., 2016; Terry et al., 2020). As these resources are depleted, some members of the primary community are displaced and a less diverse “secondary colonizing” community develops. The secondary colonizing community is specialized in the degradation of recalcitrant carbohydrates locked within a cellulose and hemicellulose matrix (Piao et al., 2014; Huws et al., 2016; Terry et al., 2020). Most studies examining feed colonization in the rumen have employed metagenomics or metataxonomic approaches focusing on changes in the makeup of the microbial community. Most recently, a study used metatranscriptomics to examine the biological function of the colonizing communities and found that microbial sub-populations co-operate, compete and rely on each other during the colonization of feed in the rumen (Huws et al., 2021). The secondary colonizing community expresses high levels of CAZymes involved in the degradation of structural carbohydrates whereas the primary colonizers display a diverse range of metabolic processes. Interestingly, the dominant microbes that are found in both stages of digestion

were observed to alter biological activity over time and displayed metabolic plasticity in response to the changes in nutrient availability that result as feed is degraded in the rumen (Huws et al., 2020). This work has shed light on the complexity of niche specialization displayed amongst rumen microbes.

Our team has examined how AFEX treatment of a variety of crop residues impacts the rumen microbial community and feed colonization in the rumen (Passetti et al., 2020; Ribeiro et al., 2020; Terry et al., 2020). Terry et al. (2020) found that colonization of crop residues was biphasic regardless of feed pretreatment with AFEX technology or fibrolytic enzymes. AFEX pretreatment was found to alter the function and composition of the primary colonizers, but not the secondary colonizers. The AFEX treated forages had an increased abundance of bacteria and pathways associated with simple carbohydrate degradation, reiterating that AFEX treatment increases the availability of a diverse range of polymers found in hemicellulose, pectin, and potentially lignin that can be rapidly metabolized by bacteria in the primary colonizing community. A related study examining AFEX treated rice straw also observed alterations in early colonization (Passetti et al., 2020). The bacterial community colonizing AFEX-treated rice straw was more similar to that colonizing high quality alfalfa hay as compared to untreated rice straw. The similarity between the microbial community colonizing alfalfa and AFEX-rice straw is reflective of the fact that AFEX increased the rapidly digestible fraction of rice straw (Passetti et al., 2020). As the duration of incubation increased the composition of phyla colonizing all forages became more similar, again illustrating that AFEX appears to alter the early stages of fibre digestion in the rumen. Ribeiro et al. (2020) examined the effect of replacing alfalfa hay with AFEX treated wheat straw on the metabolism and performance of sheep. Unlike the studies focusing on the colonizing communities, the impact of AFEX on the rumen microbiome were not as obvious. Intriguingly, the similarity between the bacteria associated with these feeds indicate that AFEX enhanced the digestibility of wheat straw to a level comparable to high quality alfalfa hay. AFEX also impacted the protozoal population in the rumen as it increased the total rumen protozoa counts in lambs as compared to an alfalfa hay diet. This observation was suggested to be due to the sensitivity of rumen protozoa to decreases in rumen pH below 5.6 (Dehority, 2003). The higher level of protozoa associated with the diet containing AFEX treated straw was attributed to a higher minimum rumen pH (5.44 vs. 5.38) and a reduction in the amount of time ruminal pH was below 5.6 (0.59 vs. 1.03 h/d) compared to the alfalfa diet (Ribeiro et al., 2020).

Digestion and Performance

Bals et al. (2010) observed that AFEX was much more effective at increasing digestion of late harvest switchgrass as compared to traditional ammoniation methods (206% vs. 56% increase, respectively). Blümmel et al. (2018) investigating the impact of AFEX on 10 different crop residues, using a ruminal batch culture in vitro method, observed that the mean apparent DM digestibility increased by 28% (493 vs 630 g/kg) after 24 h of incubation. Using the rumen simulation technique (RUSITEC) we observed that DM disappearance of barley straw improved 35% (62.4% vs. 46.2%) and total daily VFA production improved by 17.5% (58.5 vs. 49.8 mmol/d) with AFEX (Griffith et al., 2016). Similarly, AFEX treatment of wheat straw increased DM (69.6 vs. 38.3%), NDF (65.6 vs. 36.8%), and ADF (61.4 vs. 36.0%) disappearance and total daily VFA production (53.7 vs. 38.2 mmol/d) and the molar proportion of propionate, while it decreased the proportion of acetate and the acetate-to-propionate ratio in the RUSITEC (Saleem et al. 2019). These results show that the impact of AFEX treatment of cereal residues on DM and

NDF digestibility is approximately 2 times greater than that reported for traditional ammoniation of cereal straws (~15%; Fahey et al., 1993).

Beauchemin et al. (2019) evaluated the impact of AFEX on the in situ ruminal degradability of four crop residues (barley, wheat and rice straw, and corn stover). Effective ruminal NDF degradability (+28 to +126% depending upon the crop residue) was greatly increased by AFEX, mainly by increasing the potentially degradable fraction and decreasing the undigested fibre fraction. The AFEX treatment of the crop residues also increased the NDF fractional rate of degradation with the exception of barley straw, and decreased the undigested NDF (uNDF) fraction by 48 to 78% (Beauchemin et al., 2019). Passetti et al. (2020) also observed similar effects, with AFEX doubling the in situ ruminal DM and NDF degradability of rice straw after 48 h of incubation. The substantial increase in the NDF slowly degradable fraction (B) and potentially degradable fraction (A+B), and the decrease in uNDF shows the remarkable ability of AFEX to increase the proportion of NDF available for digestion in the rumen. The uNDF values observed by Beauchemin et al. (2019) for AFEX treated crop residues (i.e., <200 g/kg) were similar to values reported for high quality roughage such as alfalfa hay, corn silage and beet pulp (Soufizadeh et al., 2018). The uNDF is the NDF fraction of the diet that is not digestible by rumen microbes, even if its residency within the rumen is infinite (Huhtanen et al., 2007). As intake by ruminants fed high forage diets is often controlled by rumen fill and the rate of disappearance, a higher uNDF intake limits feed intake. The use of uNDF content of forages and diets as an indicator of physically effective NDF, gut fill, digestion, passage dynamics and intake has received increasing interest, and nutritional models have included it as an important factor in defining the intake and digestibility of forages (Ellis et al., 1999; Van Amburgh et al., 2015). Lower uNDF content of diets can decrease gut fill, increase feed passage rate through the rumen and increase intake. However, it can also decrease the physically effective fibre content and rumination time (Cotanch et al., 2014). Thus, diets containing AFEX crop residues may also need to incorporate other sources of roughages as physically effective fibre to ensure optimum rumen function and prevent ruminal acidosis.

Animal feeding trials with AFEX-treated forages are scarce. Initial studies conducted by Weimer et al. (2003) replaced 7% of the alfalfa hay in the diet of lactating dairy cows with AFEX treated rice straw and observed a 1.3 kg increase in milk production (38.3 vs. 39.6 kg/d). More recently, Mor et al. (2018) replaced wheat straw with increasing quantities of AFEX treated wheat straw pellets in a high forage diet (70% wheat straw DM basis) for lactating Murrah buffalo and Karan-Fries cattle. The AFEX diets increased cows milk production, with no changes in body weight. Buffalo fed a wheat straw diet lost body condition, while buffalo fed AFEX treated wheat straw did not. Including 50% of the diet DM as AFEX treated wheat straw pellets in the diet as substitute for untreated wheat straw increased total tract DM digestibility from 52.4 to 61.4% (17.2% increase) and milk production from 7.5 to 9.4 kg/d (25.3% increase). Mor et al. (2018) concluded that AFEX treatment of wheat straw increased the energy available for lactating dairy cattle without effecting palatability.

Passetti et al. (2020) evaluated the growth performance and digestibility of ewe lambs fed pelleted diets with 25% forage inclusion in the diet DM as alfalfa hay, rice straw, or AFEX treated rice straw. Lambs fed rice straw showed similar growth to those fed alfalfa based diets. However, lambs fed AFEX rice straw diets had lower DMI (-9%), ADG (-20%), and feed efficiency (-12%)

than lambs fed untreated rice straw diets. AFEX rice straw diets promoted similar DM but higher NDF and ADF total tract digestibility than rice straw diets. This reduction in performance with AFEX treated rice straw were not expected based on the improvements in the ruminal in situ DM and NDF degradability as a result of AFEX treatment of rice straw (Passetti et al., 2020). Untreated rice straw was expected to limit intake as a result of gut fill compared to AFEX rice straw diets. However, this was not observed likely due to the processing of the untreated rice straw, which was ground to pass through a 4 mm screen and pelleted, reducing the particle size and its residence time in the rumen. This most likely reduced the negative feedback loop on intake as a result of rumen fill, increasing the DMI and ADG of lambs fed rice straw diets.

In a second study, we looked at the effect of replacing alfalfa hay pellets with AFEX wheat straw pellets (30% of the diet DM) on the digestibility and performance of lambs (Ribeiro et al., 2020). An increase in DMI of 7.4%, and improved ADG in the first 42 d (327 vs. 304 g/d), but not over the entire feeding period (~94-d) was observed by feeding AFEX wheat straw diet compared to an alfalfa based diet (298 and 305 g/d, respectively). As a result, feed efficiency for the full feeding period was reduced (5.7%) for the AFEX straw diet compared to the alfalfa diet. Digestibility of DM was similar between diets, but digestibility of CP was reduced, and despite the higher fibre content of the AFEX diet, the digestibility of NDF and ADF increased as AFEX replaced alfalfa. There was no difference between diets in final BW as lambs were fed to a constant weight of 50 kg. However, days on feed were longer for the alfalfa-based diet than the AFEX diet (97 vs. 91 d). The fact that lambs fed AFEX wheat straw exhibited similar performance to the alfalfa diet (high quality forage), attests to its ability to improve the nutritional value of low quality forages such as straw for ruminants.

Acetamide

During the AFEX treatment of crop residues some acetamide (CH_3CONH_2) is formed by the reaction of acetyl linkages within the plant cell wall with ammonia (Chundawat et al., 2010). Acetamide is a concern as it was shown to cause liver cancer in rats when consumed at high levels for prolonged periods (WHO, 1999). Currently, acetamide can be naturally found in products such as milk, beef and thermally processed foods such as roasted coffee beans at concentrations of up to 0.4 mg/kg (Vismeh et al., 2018). Acetamide has only recently been considered a food contaminant but the maximum levels of contamination in food that are safe for human consumption have not been defined by regulatory agencies (Bals et al., 2019). Concentration of acetamide in AFEX treated cereal crop residues was shown to range from 4 to 7 mg/g of DM (Bals et al., 2019). However, the amount of acetamide in AFEX treated cereal residues are much lower than those shown to increase the incidence of liver carcinoma (23.6 mg/g of DM) in rats (Jackson and Dessau, 1961; Fleischman et al., 1980).

Acetamide is slowly metabolized by microorganisms (Arner, 1964). Mor et al. (2019) raising the possibility that ruminal bacteria could metabolize acetamide to acetate and ammonia, reducing the risk of it accumulating in meat or milk. The results from our previous study agree with this concept, as blood plasma acetamide concentrations were reduced after 2 weeks of feeding AFEX diets to lambs (Ribeiro et al., 2020). However, in our study the concentrations of acetamide in the diaphragm of lambs after slaughter were still greater for AFEX (10.1 mg/kg) compared with the control treatment (1.7 mg/kg). In a second study, we observed that acetamide in blood plasma greatly declined by removing AFEX rice straw from the diet of lambs 7 days before slaughter, but

the concentration in the diaphragm only declined slightly (2.7 vs 2.1 mg/kg; Passetti et al., 2020). These studies show that the concentration of acetamide in lamb is increased by feeding AFEX diets, although the levels are far below those that were found to cause cancer in rats (Fleischman et al., 1980).

Recombinant Fibrolytic Enzymes

Selection Process

Taking advantage of the advancements in our ability to study the rumen microbiome (e.g. non-culture techniques) and express novel recombinant enzymes, we have recently conducted a large project aiming to address some of the issues that limit the effectiveness of fibrolytic enzymes in ruminant diets. In this project we used the current knowledge of the various carbohydrate degrading enzyme families [glycoside hydrolase families (GH); i.e. families of enzymes involved in the hydrolysis and/or rearrangement of glycosidic bonds] in the rumen and our previous experience with enzyme activities associated with increased ruminal fibre degradation, to screen and select effective pure recombinant fibrolytic enzymes that would work under ruminal conditions (i.e. pH, temperature, and synergism with a mixture of ruminal enzymes). The recombinant enzymes were first selected on the basis of synergy with rumen enzymes using a high-throughput in vitro microassay (Badhan et al., 2014) that measured sugar release (glucose + xylose) when enzymes were incubated with barley straw. The best candidates were then screened using a standard ruminal batch culture technique and a semi-continuous culture system, Rumen Simulation Technique (RUSITEC), for their ability to increase the degradation of barley straw (Ribeiro et al. 2018). Based on this work we selected a recombinant enzyme that was then expressed and purified in large quantities to conduct in vivo studies with cattle (Ran et al. 2019) and sheep (Ribeiro et al. 2020).

Rumen Microbial Population

Few studies have been conducted to examine the impacts that the inclusion of enzymes in ruminant diets has on the rumen microbiome. It is possible that inclusion of enzymes may alter the availability of substrates for the rumen microbes and/or expose areas on the feed surface for colonization by rumen microbes. In our work to develop a recombinant feed enzyme adapted for the rumen environment we examined the impact of feed enzymes on feed colonization, rumen metabolism and animal performance (Ribeiro et al., 2020, Terry et al., 2020). Pretreatment of crop residues for 24 h prior to feeding did not alter the colonization process of either untreated or AFEX treated corn stover or barley straw (Terry et al., 2020). The addition of an enzyme mixture to an artificial rumen system also did not result in large shifts in the rumen microbiome; however, there was a significant change in the abundance of the bacteria *Ruminococcus flavefaciens*, and *F. succinogenes*, two important rumen fibre degrading bacteria (Saleem et al., 2019). In a study with growing lambs, the addition of a recombinant xylanase was found to alter the abundance of rumen bacteria and rumen protozoa (Ribeiro et al., 2020). It was observed that Christensenellaceae, a bacterial family with an important role in biofilm (i.e. syntrophic consortium of microorganisms that associates to each other and attaches and colonizes the surface of the substrates to promote digestion) formation and rumen degradation of starch and fibre (Mao et al., 2015; De Mulder et al., 2017), increased with enzyme treatment (Ribeiro et al., 2020). There have also been studies that have observed changes in the composition of the rumen archaeal community when enzymes

are included in the diet (Zhou et al., 2011). Fermentation of rice straw with a mixture of *Bacillus subtilis*, *Enterococcus faecalis*, cellulase, and xylanase for 14 d was observed to alter the composition of the rumen microbiome and increased acetate and propionate levels in the rumen (Hu et al 2020). The pretreatment of feeds with enzymes has been long suggested to promote the release of reducing sugars and other hydrolysis products, promoting chemotactic response that stimulate microbial attachment to feed particles (Cheng and McAllister, 1997; Beauchemin et al., 2003; Giraldo et al., 2007). We have previously observed increased bacterial colonization of feed in an in vitro rumen simulation system as a result of pretreatment of diets with enzymes (Wang et al., 2001; Ribeiro et al., 2015, 2018). Additionally, our recent work shows that the profile of bacteria attached to feed could be modified as a result of enzyme treatment (Ribeiro et al., 2020). This enzyme effect on the rumen microbial community and on the specific microbes involved in biofilm formation is still not well understood. It is likely that the enzyme and the method of enzyme application has an influence on the efficacy of treatment. The variability in the numbers and composition of the bacterial community attached to feedstuffs during ruminal digestion may help explain the variation in animal responses observed when feedstuffs are pretreated with enzymes. Future studies aimed at examining the impact of feed enzymes on ruminants should continue to evaluate their impact on biofilm formation and the rumen microbiome. A better understanding of this process may help develop enzyme treatments that can promote the attachment of ruminal microbes and enhance feed degradation in the rumen.

Digestion and Performance

In our recent work using a microassay, 1 recombinant endoglucanase (EGL7A, from GH7) and, 2 recombinant xylanases (XYL10A and XYL10C, from GH10) were selected for further evaluation in ruminal batch cultures and RUSITEC systems (Ribeiro et al., 2018). The selected enzymes consistently increased barley straw degradation in ruminal batch culture, but not in the semi-continuous culture RUSITEC system. Despite the lack of GH7 glycosyl hydrolases in the rumen, supplementation with an endoglucanase from this family did not improve the ruminal degradation of barley straw and suggested that this activity maybe redundant with other enzyme families that are present in the rumen. Only the recombinant enzyme, XYL10A, consistently improved substrate degradation in both batch culture and semi-continuous rumen fermentation systems.

Issues with scaling-up production of XYL10A made us select XYL10C the second best enzyme (best enzyme under the batch culture system) for the vivo studies using sheep and beef cattle. Supplementing the diet of beef cattle with this recombinant xylanase (XYL10C) increased in situ effective rumen degradability of AFEX treated crop residues by ~3%, but not with untreated crop residues (Beauchemin et al., 2019). The enzyme XYL10C did not improve total diet or fibre digestibility in wethers or beef heifers (Ran et al., 2019; Ribeiro et al., 2020), despite the fact that it was specifically selected for this trait in laboratory assays. XYL10C improved the ADG and G:F of lambs for the first 28 d of the performance study, but these improvements were not maintained throughout the feeding period (~94 d; Ribeiro et al., 2020). The rumen is often considered the most efficient biological system for degrading fibre (Flint et al., 2008), and making further improvements to this system presents a major challenge. The results from this lamb performance study suggest that this enzyme may promote growth in lambs early in the feeding period, but not after they were fully adapted to the diets. More studies looking at fibrolytic enzyme application into feedlot beef cattle receiving and transition diets, and in high producing lactating dairy cow diets would be interesting to test this hypothesis.

Additive Effect of AFEX and Enzymes on Feed Digestion

The combination of physical/thermochemical pretreatment and enzyme application to fibrous substrates has been used to maximize sugar release before conversion to bioethanol and also to enhance fibre digestion in ruminants (Eun et al., 2006; Wang et al., 2004; Alvira et al., 2010; Sarks et al., 2016). Using different crop residues (barley straw, corn stover, rice straw, and wheat straw), we recently evaluated the potential of AFEX treatment of these residues and the application of a recombinant xylanase (XYL10C) to improve in situ ruminal degradability (Beauchemin et al., 2019). This study demonstrated the tremendous potential of AFEX treatment to improve the ruminal degradation and the feeding value of crop residues, and that the xylanase increased the effective rumen degradability of AFEX treated crop residues by an additional 3%. The increase in the effective rumen degradability AFEX crop residues by enzyme application was due to an increase in the rate of fibre degradation, but it did not increase the total potentially degradable fibre proportion. Enzyme application did not improve the rumen degradability of the untreated crop residues. The recalcitrance of the fibre in untreated crop residues likely hindered microbial and enzyme access to substrate, thereby limiting fibre degradation. This study showed that AFEX promoted greater accessibility of the exogenous enzyme to its substrate, consequently improving fibre digestion (Beauchemin et al., 2019). This work also shows the potential of combining these technologies to maximize the ruminal degradation of these feed residues.

Future Challenges and Conclusions

Although we have shown that AFEX technology can greatly improve the digestion and the feed value of low quality crop residues there is still much work to be done to scale up this technology to a commercial level. Developing central industrial facilities that can receive and process crop residues and produce AFEX treated feedstuffs in a cost effective manner presents a challenge, but the increased use of forages could increase the sustainability of the ruminant livestock industry.

Development of recombinant fibrolytic enzymes specific for ruminant diets has proven to be a challenging task. Our work suggests that the use of fibrolytic enzymes may have greater success in the earlier stages of the feeding period (< 30 d) when the ruminal environment is not fully adapted to the diets. The ruminal microbial community has evolved over millions of years and due to its redundancy, resilience, and ability to adapt to different feedstuffs, the development of new enzymes that can further improve ruminal fibre digestion is not a simple task. New recombinant rate limiting fibrolytic enzymes may only be effective until the next rate limiting enzyme starts to impede fibre digestion. A greater understanding of the ruminal microbial biofilm and the attachment process, and how enzymes could be used to manipulate this process may be a way to increase the efficiency of enzyme-based technologies in the future.

Combining different technologies to improve fibre digestion, like AFEX and recombinant enzymes, can maximize the energy extraction from recalcitrant fibre sources and likely improve the productivity, profitability, and sustainability of ruminant livestock systems.

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Diurnal Lighting Programs for Broilers – Do They Impact Broiler Behaviour and Feed Efficiency?

Les programmes d'éclairage diurne pour les poulets à griller : peuvent-ils influencer sur les comportements alimentaires et l'efficacité alimentaire?

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Abstract

The use of diurnal cycles (day and night periods within a 24 hr timeframe) are not universally used, and in those cases, constant (24 hours of light with no dark period (24L: 0D)) or near-constant (23 hours of light and 1 hour of dark (23L:1D)) are instead utilized. The use of diurnal patterns alters bird behaviour, and results in more active broilers, with a higher percentage of time spent in comfort and exploratory behaviours that clearly indicate positive welfare. The use of dark also results in changes to feeding behaviour. In cases when birds have learned that darkness will come at a certain time each day, they will consume a large amount of feed prior to onset of darkness. Often, this occurs approximately an hour prior to lights off. This peak in feed activity is very important, as it allows the bird to consume enough feed to maintain contents in the gastrointestinal tract throughout the majority of the dark period. Utilizing longer hours of darkness results in a larger amount of feed consumed prior to darkness, and, regardless of the length of that dark period (up to 10 consecutive hours), the gastrointestinal tract empties at approximately the same amount of time prior to lights on. This, along with increased weight of organs such as the gizzard, are likely contributing factors to the improved feed efficiency noted for broilers on a lighting program with a minimum amount of 4 hr of dark.

Résumé

Les cycles diurnes (périodes de jour et de nuit dans un intervalle de 24 heures) ne sont pas universellement utilisés et, dans ces cas, on applique plutôt des cycles constants (24 heures de lumière sans période d'obscurité [24L:0D]) ou quasi-constants (23 heures de lumière et 1 heure d'obscurité [23L:1D]). L'utilisation de schémas diurnes modifie le comportement des oiseaux et se traduit par des poulets à griller plus actifs chez qui les comportements de confort et d'exploration occupent plus de temps, soit un signe clair de bien-être. L'usage de l'obscurité entraîne également des changements dans le comportement alimentaire. Les oiseaux qui finissent par apprendre que l'obscurité tombera à une certaine heure chaque jour consomment une grande quantité de nourriture avant l'arrivée de la nuit. Souvent, cela se produit environ une heure avant l'extinction des lumières. Ce pic d'activité alimentaire est très important, car il permet à l'oiseau de consommer suffisamment d'aliments pour maintenir le contenu du tube digestif pendant la majeure partie de la période

d'obscurité. Une plus longue durée d'obscurité entraîne la consommation d'une plus grande quantité d'aliments avant la tombée de la nuit et, quelle que soit la durée de cette période d'obscurité (jusqu'à 10 heures consécutives), le tube digestif se vide approximativement au même moment avant l'allumage des lumières. Ce phénomène et l'augmentation du poids des organes tels que le gésier sont probablement des facteurs qui contribuent à l'amélioration de l'efficacité alimentaire constatée chez les poulets à griller élevés avec un programme d'éclairage comportant une période d'obscurité minimale de 4 heures.

Introduction

Recommendations for lighting programs during the rearing period for broilers generally include a dark period. In Canada, the Codes of Practice for Chickens, Turkeys and Breeders (https://www.nfacc.ca/pdfs/codes/poultry_code_EN.pdf) require that a minimum of 4 hours of continuous dark is included in a photoperiod program from day 5 of placement to no sooner than 7 days prior to catching. However, this remains a controversial subject, and the inclusion of darkness is not included in all programs world-wide.

The published data supports the positive impacts of allowing a dark period for sleep on numerous aspects of a broiler production program. For example, growth rate and feed conversion are improved, health is improved, diurnal rhythms function as they should, behavioural expression, including the expression of positive behaviours, is broader. These responses result in significant improvements in productivity and bird welfare.

Review

The traditional thought pattern involved providing broilers continuous (24 hours of light (24L)) or near-continuous (23 hours of light and 1 hour of dark (23L:1D)) light to allow them near-constant visual access to feed and watering systems, and the ability to move throughout the barn facility as they wish. With that thought pattern, a common belief was that 23L:1D or 24L:0D would result in heavier body weights at the time of marketing compared to those broilers reared with the utilization of a dark period. However, numerous research projects have clearly indicated that this is not the actual response. In our work (Schwean-Lardner et al., 2012a), we found that as early as 32 days of age, broilers were heavier at market age when allowed 4 hours of consecutive darkness compared to 23L:1D, and by 49 d of age, heaviest weights were found when broilers were reared under 17L:7D. At this age, allowing 10 hr of darkness for broilers actually resulted in numerically heaviest weights compared to birds reared under 23L. In addition, feed efficiency improved with the addition of darkness, so not only can producers improve body weights with a simple addition of darkness in a photoperiod program, but can also reduce feed purchases through improved feed conversion ratios. The addition of darkness in a photoperiod program also improves the health of broilers, reducing mortality and morbidity. The differences generally are noted with respect to both skeletal and metabolic related diseases (Schwean-Lardner et al., 2013). Other health improvements are noted in reduced footpad dermatitis lesions, and significant improvements in bird mobility (Schwean-Lardner et al., 2013). The addition of darkness also changes bird behaviour. Birds given a minimum of 4 hr of darkness spend more time performing positive behaviours such as stretching, playing and preening, move in their environment more and spend more time at the feeder than do birds on continuous or near-continuous photoperiods (Schwean-Lardner et al., 2012b).

How does this Impact Feed Efficiency and Gut Transit?

Much of the earlier data from this lab did not determine what the mechanisms are that can impact feed efficiency when longer periods of darkness are provided for broilers. However, recent work by Shynkaruk et al. (2019) may have an answer. Their work found that if broilers know when darkness is coming, feeding patterns are adjusted to include an anticipatory feeding prior to lights off. By doing this, they maintain feed in the gastro-intestinal tract for the majority of the dark period.

With respect to the feeding behaviour, birds increase the number of times they go to the feeder during the photophase in a linear manner. In other words, they have more meals, and more drinking bouts, during the photoperiod every hour that the lights are on (Table 1).

The feeding activity increases approx. 30-120 min before lights go off (Schwean-Lardner et al., 2014). This additional feed intake results in birds with heavier crops and gizzards (Shynkaruk et al., 2019; Table 2), which may improve their function, resulting in improved digestibility of feedstuffs.

Regardless of the time of lights off, the crops and gizzards of birds on each of these lighting programs emptied at approximately the same time, just prior to lights returning on for the following day (Figure 1 and 2). This suggests that retention time of feedstuffs is longer in the digestive tract, which may also improve feed efficiency for these birds. Classen et al. (2016) reported that enzymes found in feed, water, saliva or other microorganisms can initiate digestion in the crop, and that this may be more effective if the feedstuff remains there for a longer period of time. A longer retention time in the gizzard, as well as a more muscular gizzard, can also increase digestibility of feedstuffs (Table 2). The remaining gastrointestinal tract showed similar trends in emptying, with each section reaching near-empty at approximately the same time before lights come on regardless of the lighting program tested.

In conclusion, the latter study (Shynkaruk et al., 2019) has provided evidence that birds anticipate darkness (when a set dark onset is used) and eat accordingly to maintain feedstuffs throughout the gastrointestinal tract until just prior to lights on. The gut is not completely empty at any time. This increased time, along with indications of increased functioning of the crop and gizzard, may at least in part explain the improved feed efficiency when diurnal photoperiods are used for broilers. The increased feed efficiency, along with reduced mortality and heavier body weights at market age, should result in increased profits for producers.

Table 1. Effect of darkness on the average number of feeding and drinking bouts in broiler chickens (average of males and females at wk 2 and 4) (Shynkaruk et al., 2019).

Item	Hours of darkness				SEM	Regr ¹ :P ²
	1	4	7	10		
Photoperiod						
Feed bouts/hour	3.1	3.3	3.4	4.3	0.15	L:0.05
Drink bouts/hour	2.8	3.6	4.5	5.6	0.22	L:0.03

¹Analysed with Regression Analyses, SAS, where L refers to linear relationship; ² Probability of difference.

Table 2. Effect of darkness on empty crop and gizzard weight and content expressed as a percentage of body weight (Shynkaruk et al., 2019).

	Hours of darkness					
Item	1	4	7	10	SEM	Regr ¹ :P ²
Empty weight						
Crop	0.27	0.24	0.30	0.33	0.005	Q:<0.01
Gizzard	1.36	1.39	1.42	1.49	0.012	L:<0.01
Content weight						
Crop	0.44	0.46	0.65	1.14	0.048	L:<0.01
Gizzard	0.80	0.84	0.84	1.00	0.026	L:<0.01

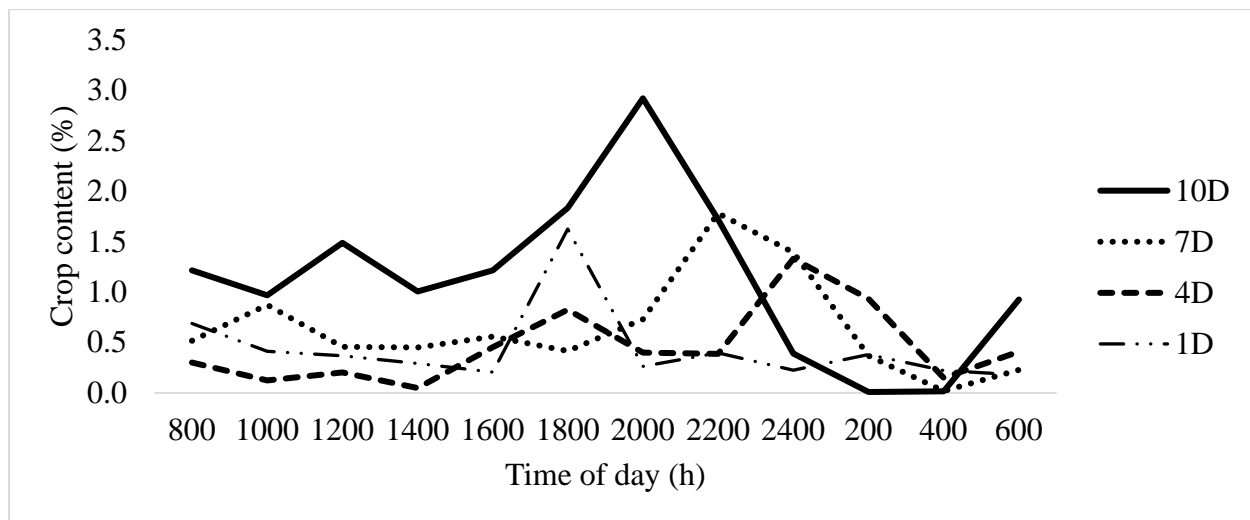


Figure 1. Effect of dark exposure and time of day on crop content expressed as a percentage of body weight. The dark periods corresponding to the lighting treatments are as follows: 2000 to 600 (10D), 2300 to 600 (7D), 200 to 600 (4D), and 500 to 600 (1D).

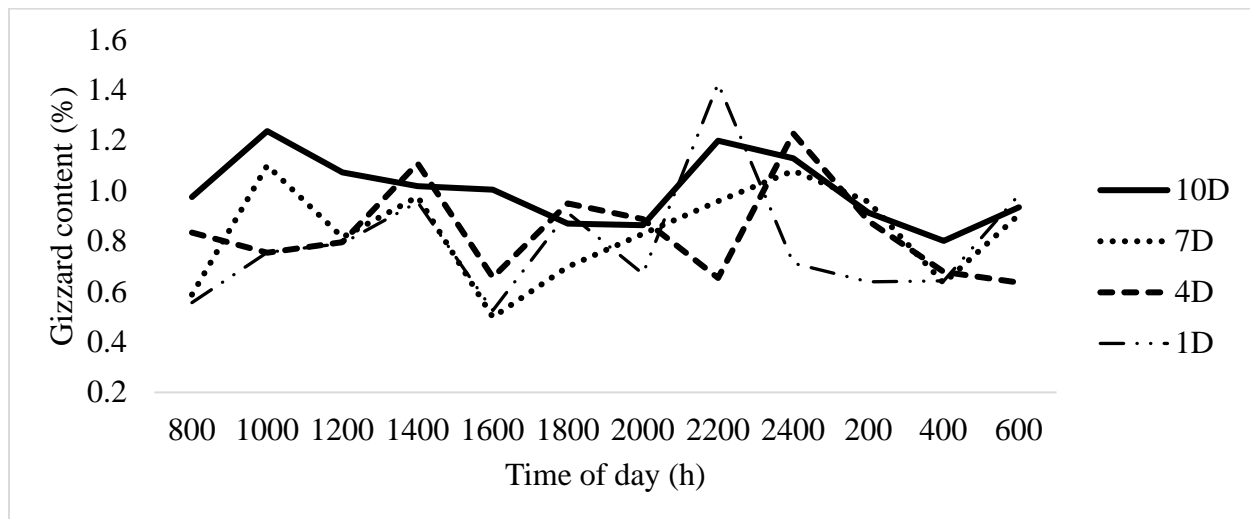


Figure 2. Effect of dark exposure and time of day on gizzard content expressed as a percentage of body weight. The dark periods corresponding to the lighting treatments are as follows: 2000 to 600 (10D), 2300 to 600 (7D), 200 to 600 (4D), and 500 to 600 (1D).

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Precision Determination of Energy and Protein Requirements of Grazing and Feedlot Animals

Détermination avec précision des besoins en énergie et en protéine des animaux au pâturage et en parc d'engraissement

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Abstract

The energy expenditure (**EE**) required for walking (i.e., locomotion), body movements, and forage browsing, selection, and prehension for grazing animals is likely the main difference between pasture-raised and feedlot-raised ruminants, assuming that the dietary metabolizable energy is similar between these systems. However, our incomplete understanding of these components keeps rebounding time after time without a more definitive solution. Different techniques were developed during the early 1960s throughout the mid-1980s to assess grazing animals' EE, but they are far from ideal. The majority of the data on ruminants' EE climaxed during the same period, boosted by many open-circuit, indirect calorimetry apparatuses and methods. However, a considerable variation exists when literature data is analyzed because many factors can alter the EE of grazing animals. New data and novel predictive analytics are indispensable to propel our scientific understanding of plant and animal interactions (e.g., forage selection, grazing behavior, pasture growth/regrowth, pasture quality, nutrient digestion and absorption, volatile fatty acids production and profile, energy requirement). The scientific community has been under a spell since the mid-1980s, and only recently, in the last five years, we have been invigorated by the advance of sensors (and artificial intelligence) that has allowed us to embark on a gradual crescendo of excitement. We must continue to boost the investment in non-invasive techniques onwards; otherwise, we will once again paralyze our understanding of energy needs by grazing ruminants, jeopardizing our search for sustainable livestock production, and upsurge our dependability on feedlots to produce meat to satisfy the population demand.

Résumé

La dépense énergétique (**DE**) associée à la marche (c'est-à-dire la locomotion), aux mouvements du corps ainsi qu'à la recherche, à la sélection et à la préhension du fourrage chez les animaux au pâturage est probablement la principale différence entre les ruminants élevés au pâturage et ceux élevés en parc d'engraissement, en supposant que l'énergie alimentaire métabolisable est similaire entre ces systèmes. Cependant, notre compréhension incomplète de ces éléments refait continuellement surface sans qu'une solution plus définitive soit trouvée. Différentes techniques ont été mises au point au début des années 1960 et jusqu'au milieu des années 1980 pour évaluer la DE des animaux au pâturage, mais elles sont loin d'être idéales. La majorité des données sur la

DE des ruminants ont été recueillies durant la même période, consécutivement à l'apparition de nombreux appareils et méthodes de calorimétrie indirecte en circuit ouvert. Cependant, l'analyse des données de la littérature révèle une variation considérable, car de nombreux facteurs peuvent modifier la DE des animaux au pâturage. Nous devons indiscutablement recourir à de nouvelles données et techniques novatrices d'analytique prédictive pour alimenter nos connaissances scientifiques sur les interactions entre les plantes et les animaux (par exemple, la sélection des fourrages, le comportement au pâturage, la croissance/repousse des pâturages, la qualité des pâturages, la digestion et l'absorption des nutriments, la production des acides gras volatils et leur profil, les besoins énergétiques). La communauté scientifique est au ralenti depuis le milieu des années 1980, et ce n'est que récemment, au cours des cinq dernières années, que nous avons été revigorés par les progrès des capteurs (et de l'intelligence artificielle) qui nous ont permis de connaître une montée d'enthousiasme. Nous devons continuer à favoriser l'investissement dans les techniques non invasives, à défaut de quoi notre compréhension des besoins énergétiques des ruminants au pâturage sera à nouveau paralysée, compromettant ainsi les efforts de recherche visant la durabilité de la production animale, et nous renforcerons notre dépendance vis-à-vis des parcs d'engraissement pour produire la viande nécessaire afin de satisfaire la demande.

Introduction

The United Nation's sustainable development goals¹ aim to "...achieve a better and more sustainable future for all" by addressing "...the global challenges we face, including poverty, inequality, climate change, environmental degradation, peace and justice." These challenges are interconnected and depend on integrating innovative ideas of several science fields to achieve sustainability, especially in livestock production systems. The perfect match between what animals require to perform as-desired and what dietary ingredients supply to them is a *sine qua non* condition to sustainably meet animal products' demand by the human population worldwide. It is a wicked problem to be solved in many ways, and solving it requires a systems approach (Tedeschi et al., 2015). Grazing and feedlot systems are two completely different production conditions for ruminants, and they are not necessarily mutually exclusive. Sometimes the grazing phase precedes the feedlot phase, and, for some regions in the world, it can be one or the other for the complete production duration (Cottle and Kahn, 2014).

The majority of recommendations for grazing animals' energy and protein requirements have been determined on confined animals that are much closer to animals' requirements raised under feedlot conditions. In part, the determination of energy and protein, as well as other nutrients, for grazing animals require special equipment and methodology that makes it more challenging, expensive, and laborious. On top of that, such estimates' variability is much greater for grazing than confined animals because grazing animals are prone to the impact of additional environmental factors that confined animals are not. The requirements of energy and protein for confined animals have been extensively studied and disseminated for over 116 years (Kellner, 1905), using concepts devised in the late 1700s by many illustrious scientists, including Leonardo da Vinci¹⁴⁵²⁻¹⁵¹⁹, Joseph Priestley¹⁷³³⁻¹⁸⁰⁴, Carl Wilhelm Scheele¹⁷⁴²⁻¹⁷⁸⁶, Antoine-Laurent Lavoisier¹⁷⁴³⁻¹⁷⁹⁴, Pierre-Simon Laplace¹⁷⁴⁹⁻¹⁸²⁷, and Justus Freiherr von Liebig¹⁸⁰³⁻¹⁸⁷³ (Tedeschi and Fox, 2020a, Ch. 4). Digestion trials have been implemented for a bit longer: since 1860 at the Weende Experiment Station at the

¹ <https://www.un.org/sustainabledevelopment/sustainable-development-goals/>

University of Goettingen in Germany, and since 1884 at the University of Wisconsin Agricultural Experiment State in the United States (Schneider and Flatt, 1975).

Many classical books (Baldwin, 1995; Blaxter, 1962; Brody, 1945; Kleiber, 1961) and literature reviews (Ferrell and Oltjen, 2008; Garrett and Johnson, 1983; Johnson et al., 2003; Tedeschi, 2019a) have comprehensively discussed the transactions of animal bioenergetics. For beef cattle, currently recommended energy and protein requirements stemmed from pen-fed animal studies using the comparative slaughter technique and the California Net Energy (**NE**) System methodology (Oltjen, 2019), though discrepancies might exist (Tedeschi et al., 2017; Tedeschi, 2019b). For dairy cattle, current recommendations for energy and protein requirements are based on the indirect calorimetry using open-circuit respiration chambers (Flatt et al., 1965a, b; Moe et al., 1972; Moe, 1981) at the Energy Metabolism Laboratory at the Dairy Cattle Research Branch, United States Department of Agriculture, Agricultural Research Center, Beltsville, Maryland, US (Flatt et al., 1958). Most modern requirement studies were initiated in the 1960s worldwide.

Grazing animals have an additional energy requirement associated with the grazing activity compared to confined animals' requirements. It comprises the additional energy needed for body movements (i.e., locomotion) and forage browsing, selection, and prehension. The non-activity maintenance requirement of energy between grazing and confined growing or finishing animals might be identical on a metabolic weight basis, as long as animals are at the same maturity degree (i.e., same composition of gain) (Tedeschi and Fox, 2015). However, because the diet consumed by grazing animals (i.e., essentially forage) have a lower partial efficiency of energy use for growth (**k_g**), grazing animals would require a greater dry matter intake (**DMI**) to achieve the same average daily gain (**ADG**). This fact becomes a significant limitation for grazing animals for two main reasons: **1**) the distance traveled to reach maximum voluntary intake within a 24-h period (daily basis) may exceed animal's locomotion capacity, worsening its energy balance; and **2**) because DMI is also a function of rumen size (i.e., volume/space), low-quality forages, i.e., forage containing a higher proportion of fibrous material, may further restrict intake by triggering the negative effect of rumen fill on voluntary feed intake (Tedeschi and Fox, 2015; Tedeschi and Fox, 2020a, Ch. 10). Both reasons concurrently impede grazing animals to have the same ADG as confined animals. Although the energy cost of physical activities in cattle, sheep, and buffaloes has been extensively documented worldwide, a comprehensive physical activity calculation logic does not exist because the lack of information on the energy required for consuming and processing feed by the animal is considerably larger for grazing animals than for confined animals (Tedeschi and Fox, 2015).

This review's main objectives are to **1**) illustrate different methods to estimate feeds' nutritive value, **2**) briefly discuss existing techniques and methods to assess energy expenditure (**EE**) in grazing and confined animals, and **3**) provide a different calculation logic in predicting energy requirement for grazing animals.

Determination of Feed Nutritive Value

An essential step in determining energy and protein requirements is to have an accurate assessment of the nutritional value of the feeds consumed by the animals. This is not a trivial step. Based on

wet gravimetric chemistry methodology, chemometric techniques are the preferred methods to determine nutrient contents in feeds (Faithfull, 2002; Moughan and Hendriks, 2018; Van Soest, 2015), and when associated with animal's *in vivo* digestibility trials (Schneider and Flatt, 1975), an assessment of digestible energy (**DE**) and total digestible nutrients (**TDN**) becomes possible. In essence, chemometric techniques are used for critical nutrients (e.g., protein, ether extract, and carbohydrate) in determining the “energy content” of feeds through DE or TDN. For practical purposes, in ruminants, 1 kilogram of TDN is assumed to contain 4.4 kcal of DE (Swift, 1957). Although energy is defined as “the ability to perform work,” different “forms of energy” have been defined, depending on how it is used (or lack of) by the animal. The energy content of a feed (or feed's nutrient) is determined using adiabatic or ballistic bomb calorimeters (McLean and Tobin, 1987), and it is referred to as gross energy (**GE**).

Upon consumption (and rumination) by the animal, the digesta undergoes many physicochemical transformations in the gastrointestinal tract, including fermentation in the rumen and hindgut, and enzymatic digestion in the midgut, before being absorbed. The amount of DE is computed by difference based on the GE contents in the feed and feces, $DE = (Feed\ Intake \times GE_{Feed} - Feces \times GE_{Feces}) / (Feed\ Intake \times GE_{Feed})$. In ruminants, the amount of energy available to the animal is called metabolizable energy (**ME**), and it is computed after removing the energy lost to gas production in the rumen (methane, **CH₄E**) and urine substances (**UE**), $ME = DE - (CH_4E + UE)$. Finally, ME is further adjusted to the heat production (**HP**) depending on its fate (e.g., maintenance, tissue deposition, milk production) (NASEM, 2016; NRC, 2001). Though this sketch is an oversimplification of factors involved in the animal's bioenergetics, it still provides a general idea of the feeds' supply of energy.

Energy is an attribute of the feed that results from the combination of different nutrients and their digestibility and metabolism by the animal. Hence, it is interesting to note that plant defensive compounds (**PDC**) (Tedeschi and Fox, 2020a), also known as antinutritional factors, are not explicitly considered when determining TDN or DE of feeds, likely because it is assumed that such compounds would exert their negative impact during the *in vivo* digestibility trial; thus, already decreasing the digestibility. However, the conditions in which animals consume PDC-containing feeds might differ from those used to determine their TDN, especially under grazing conditions when plants might be at various growth stages and contain different types and concentrations of PDC during the growth of new leaves (Harborne, 1993).

Techniques to Determine the Nutritive Value of Feedstuffs

Wet chemistry has been the golden standard for determining the chemical composition of feeds and, ultimately, their nutritive value. Nevertheless, there are limitations such as the cost of the analyses, the time it takes to obtain the results, and the labor for collecting representative samples and processing them for subsequent analyses that prevent broad adoption of wet chemistry. Perhaps the main problem happens with grazing animals. A representative sample of what the animal is consuming is practically impossible to obtain, and the speed the forage changes its physicochemical characteristics is likely faster than the obtention of the laboratory results. Thus, alternative, quicker techniques have to be employed.

Near-infrared spectroscopy (**NIRS**) resulted from Sir Frederick Herschel's pioneering work, who studied the infrared radiation while passing sunlight through a prism and measuring its

temperature. Spectroscopy and spectrography were born in the 19th century, and circa the 1960s (Shenk and Westerhaus, 1994), scientists started adopting spectrophotometric techniques given their automation in the laboratory and high correlations with some forage components measured via wet chemistry (Norris et al., 1976). For grazing systems, NIRS provided opportunities that did not exist or were very difficult to achieve through wet chemistry, including daily flux of diet quality consumed by grazing animals (Lippke and Barton, 1988; Stuth et al., 1991; Stuth et al., 2003). However, limitations also exist. Quicker methods may be less reliable and prone to the need for constant cross-validation and calibration (Stuth et al., 1991). Real-time NIRS has been used to determine pasture nutritive composition (Bell et al., 2018) and quality (Serrano et al., 2020), and when associated with fecal scans, pasture nutritional profiling for grazing animals can be predicted (Dixon and Coates, 2009; Stuth et al., 2002).

Another novel spectroscopic technique is Raman microscopy (or spectroscopy) with laser excitation (Delhaye and Dhamelincourt, 1975; Landsberg and Imandelstam, 1928; Raman and Krishnan, 1928). It is based on measuring the degree of energy loss due to light scattering associated with different chemical molecules' fingerprints, allowing for their identification in addition to revealing their spatial distribution within the sample at micrometer or nanometer scales. Raman spectroscopy has a wider spatial resolution than NIRS, and it can operate through water and glass (De Gelder et al., 2007). It also requires fewer preparatory steps of the sample, making it faster and easier to use in field conditions. For example, along with chemometric methods, a handheld Raman spectrometer device was used to determine the contents of carbohydrates, fibers, carotenoids, and protein in corn grain as accurate as NIRS' predictions (Krimmer et al., 2019). Raman spectroscopy has assisted in genotyping high-starch containing potato (Morey et al., 2020) and peanut (Farber et al., 2020) and in identifying the nutritional quality of sweet cream butter of dairy cows fed different total mixed diets (Gómez-Mascaraque et al., 2020).

The literature seems scarce or inexistent about using Raman spectroscopy to determine the nutrient composition of forages, mostly pasture composed of mixed forages such as grasses and legumes. Similarly, there is no concrete resolution on the use of Raman or NIRS to determine PDC. Regardless of the lack of definitive literature on the application of Raman or NIRS to estimate the chemical composition of forages (or any other feedstuff), it is not clear if the precision and accuracy obtained with these techniques and current devices are sufficient to reliably estimate energy consumption, more specifically DE or TDN, to assess energy requirement of grazing and confined animals.

Both physicochemometric analytical methods and spectroscopic techniques provide a snapshot of the feed's nutrient content. There have been reports that spectroscopic techniques (e.g., NIRS) can adequately assess nutrient contents, i.e., pool sizes, but they cannot reliably and repeatedly estimate abstract measurements such as digestibility (Coates and Dixon, 2011). Furthermore, there are incoherences in using NIRS to determine digestibility between *in vivo* and *in vitro* systems (Mahipala et al., 2010). In part, spectroscopic techniques cannot inform rumen fermentation dynamics because many factors, other than feed composition, are involved in the digestion process. They may correctly appraise the pool size, but they reveal nothing about what happens in the ruminant animal's gastrointestinal tract; unless multiple snapshots are taken throughout the digestive process, which is nearly impossible. Consequently, feeds with identical physicochemical composition may degrade differently in the rumen for reasons not unveiled by wet chemistry

procedures or spectroscopic techniques. Schneider and Flatt (1975) go one step further by affirming that “two feeds may be equal in composition and equally digestible, yet one may be more valuable than the other because its digested matter can be used to better advantage by the body.” Alternatively, researchers need to reliably measure the same pool size in the same animal’s feces to compute DE by difference like digestibility trials. However, researchers rarely can track down the same animal under pasture conditions to collect feces, let alone collecting the feces that represent the consumed forage.

Approaches using *in vitro* techniques as a proxy for *in vivo* digestibility have been suggested, such as the original or modified two-stage digestion proposed by Tilley and Terry (1963) or the gas production-based techniques originally proposed by Menke et al. (1979). However, limitations also exist, including the lack of confirmation that and when *in vitro* can represent *in vivo* digestibility (Tedeschi and Fox, 2020a).

Finally, given our inability to definitively and accurately assess the consumption of DE of animals when a digestibility trial cannot be carried out, some have proposed the use of mathematical modeling or empirical predictions to predict DE or TDN given the chemical composition of the diet in addition to other factors (Tedeschi and Fox, 2020a, b), especially for those under grazing conditions (Tedeschi et al., 2019; Woli et al., 2020). In part, the problem arises not only because the DE content is not known with a high degree of certainty but because of inadequate feed intake predictability even though many models for such prediction abound in the literature (Tedeschi et al., 2019). The question persists even when using mathematical models: can the intake of DE be accurately determined so energy partitioning can be estimated to assess animals’ EE at grazing or confined conditions? In other words, how confident can one be in using wet chemistry, NIRS/Raman, or modeling to estimate DE or TDN? This conundrum applies to grazing and confined animals when wet chemistry and digestibility trial cannot be conducted.

Determination of Energy Expenditure

As indicated above, the comparative slaughter technique has been the preferred method to determine the energy and protein requirements of growing animals, whereas indirect calorimetry has been used for lactating dairy cows. Regardless of the technique used to determine animals’ EE, they must follow the first law of thermodynamics in which energy can neither be created nor destroyed but instead transformed from one form to another. Therefore, based on the first law of thermodynamics, we assume the intake of ME is equal to the sum of heat produced and energy retained (or excreted, e.g., milk) by the animal. This assumption is fundamental to any technique used to determine the EE by animals.

In the United States, comprehensive discussions about energy and protein requirements, mostly based on confined animals (Tedeschi, 2019a), have been published by the National Research Council (**NRC**) and National Academies of Sciences, Engineering, and Medicine (**NASEM**) for beef cattle from 1945 (NRC, 1945a) to 2016 (NASEM, 2016), for dairy cattle from 1945 (NRC, 1945b) to 2001 (NRC, 2001), for sheep and goats from 1945 (NRC, 1945c) to 2007 (NRC, 2007), as well as other publications (Cannas et al., 2004; Tedeschi and Fox, 2020a, b). Other countries and regions around the globe have followed suit and devised their own set of recommendations to

meet their needs and production conditions. Few publications have addressed the grazing animal requirement meticulously as the Australian Nutrient Requirements of Domesticated Ruminants (CSIRO, 1990; 2007).

Comparative Slaughter Technique

The comparative slaughter technique consists of measuring the body energy at the beginning and the end of a feeding trial (usually longer than 30 days), using *precisely* identical animals to determine their retained energy (**RE**) and protein (**RP**). Because no two animals are *precisely* identical (except for monozygotic twins, genetically speaking), many animals are needed to achieve adequate representation, but unfortunately, hardly attained. Significant concerns have been raised about **1**) incoherent relationships between predicted and observed RE and RP, and **2**) the calculation logic for k_g derived from diet characteristics rather than carcass composition (Tedeschi et al., 2019) besides other inconsistent-prone ancillary calculations that support the comparative slaughter technique (Tedeschi et al., 2017), including adequate sample size. Although the comparative slaughter technique provides a direct assessment of RE and RP, it is an expensive method due to sampling problems; thus, been recommended for small animals (Blaxter, 1967). In the comparative slaughter technique, ME intake and RE are measured, and HP is computed by difference.

Calorimetry Techniques

On the other hand, calorimetry measures the HP produced by animals and computes RE as the difference between ME intake and HP. Two types of calorimetry exist: direct or indirect.

Direct calorimetry techniques measure the heat emission from the calorimeter and its contents or occupants during a period through changes in the water's temperature surrounding the calorimeter. It relies on the heat balance equation in which heat elimination is the sum of heat lost by radiation, convection, conduction, and evaporation. Direct calorimetry is hardly used to determine an animal's energy requirement because of its costs and laborious management, making its use even harder for grazing conditions. In animal science, direct calorimetry finds its way in measuring feed's GE with adiabatic or ballistic bomb calorimeters (McLean and Tobin, 1987).

Indirect calorimetry techniques determine heat production by quantifying the consumption of O_2 and the production of CO_2 and CH_4 and the excretion of urinary N (Gerrits and Labussière, 2015; McLean and Tobin, 1987). Besides the measurements of HP, indirect calorimetry can also assist in nutrient assimilation, thermogenesis, the pathogenesis of obesity and diabetes, and energetics of physical exercise (Ferrannini, 1988). Indirect calorimetry relies on the principle of conservation of energy (i.e., the first law of thermodynamics), and it can be either closed- or open-circuit indirect calorimetry. In the closed-circuit indirect calorimetry, CO_2 is removed from the air inside the respiration chamber using a potassium hydroxide solution, and O_2 is provided from a volumetric flask (McLean and Tobin, 1987). The open-circuit indirect calorimetry measures the volume and gaseous composition of the air coming in and out of the respiration chamber (McLean and Tobin, 1987), and O_2 consumption and production of CO_2 and CH_4 are estimated by difference. The validity of indirect calorimetry relies on the proven assumption that O_2 uptake by the animal can be estimated by the difference between the inflow and outflow of O_2 (Nienaber et al., 1993). The open-circuit indirect calorimetry is more amenable to determine the EE of grazing animals.

The investigations on the calculations of energy metabolism by a sub-committee on constants and factors appointed at the First Energy Metabolism Symposium in 1958 led to the development of an empirical equation to estimate HP based on an animal's gaseous exchange from closed-circuit calorimetry (Brouwer, 1965), as shown in Eq. [1]. For open-circuit calorimetry, McLean (1972) proposed Eq. [2], which is based on Eq. [1] after adjusting for the composition of inlet air (20.95% O₂ and 0.03% CO₂).

$$HP = 3.866 \times O_2 + 1.2 \times CO_2 - (0.518 \times CH_4 + 1.431 \times N) \quad [1]$$

$$HP = V_0 \times (4.891 \times \Delta O_2 + 0.175 \times \Delta CO_2 - 1.543 \times \Delta CH_4) - 1.431 \times N \quad [2]$$

Where CH₄ is methane production, L; CO₂ is carbon dioxide production, L; HP is heat production, kcal; N is urine-N, g; O₂ is oxygen consumption, L; ΔCH_4 is the difference of CH₄ between the inlet and outlet air compositions, L; ΔCO_2 is the difference of CO₂ between the inlet and outlet air compositions, L; and ΔO_2 is the difference of O₂ between inlet and outlet air compositions, L.

There are several techniques based on the open-circuit indirect calorimetry that can be used in determining the EE of grazing animals, including:

1. The portable mask technique, sometimes referred to as mobile indirect calorimetry (Lachica and Aguilera, 2008), uses an airtight facemask fitted with inlet and outlet valves and a flow meter to determine breath volume and composition, and EE is computed based on the differences of gas volumes after adjusting for temperature, pressure, and humidity (Lawrence et al., 1991). Limitations with this technique include **1)** slow or lagging O₂ sensor responses, **2)** water vapor condensation inside the facemask, and **3)** animals do not habituate with the facemask and have undesirable behavior, including animal's panting. Tracheal cannulas have partially replaced portable facemasks (Whitelaw, 1974). However, problems still exist, such as adequate sample size to adequately determine EE and the impact of the technique on the animals' normal grazing behavior.
2. Portable troughs containing a headstall unit to restrict and control the atmospheric mixing of gases using a gas manifold and sensors to measure the airflow and its composition (e.g., CO₂ and CH₄) have been used in recent years (Zimmerman et al., 2011). The main application of this partial open-circuit indirect calorimetry has been to estimate CH₄ emission of cattle herd (Alemu et al., 2017; Huhtanen et al., 2019; Waghorn et al., 2013) and, more recently, EE via CO₂ output (Caetano et al., 2018). Pending additional validation of this technique on a large scale, the benefits are incredible because it can measure gaseous exchanges of many animals at different times of the day, overcoming the variations of short-term measurements and mimicking the measurements obtained with respiration chambers (Hegarty, 2013).
3. The labeled (or heavy) water technique proposed by Lifson and McClintock (1966) is an isotopic non-radioactive method that administers deuterium (D = ²H) and oxygen-18 (¹⁸O) into an animal and measures the elimination of D₂¹⁸O (heavy water) and C¹⁸O₂. The rate of CO₂ production can be estimated through isotopic enrichment difference between consecutive measures of urine samples. For instance, an animal given a dose of water (D₂¹⁸O) will reduce the specific activity of the O₂ in the body faster than that of D. The rate of C¹⁸O₂ production is computed as the difference in these two rates multiplied by the volume of the total initial body water content (Lawrence et al., 1991). Several adjustments have to be made before estimating the EE (Fancy et al., 1986; Haggarty, 1991; Nagy, 1980) that make this technique laborious, let alone the expenses involved in producing the

isotope-enriched water and highly sophisticated analytical techniques needed to determine the isotope concentration.

4. The carbon dioxide entry rate technique was initially used to estimate the EE of grazing sheep (Young et al., 1969) and cattle (Young, 1970). This is an exciting technique that relies on continuous CO₂ production and excretion, leading to a CO₂ pool in the body, and a constant infusion of ¹⁴CO₂ (e.g., 20 nCi/min of NaH¹⁴CO₃ in an aqueous solution) until an equilibrium concentration in the excreted CO₂ is reached. Such equilibrium depends on the rate of infusion of NaH¹⁴CO₃ and the elimination of CO₂ produced endogenously (Lawrence et al., 1991; Whitelaw, 1974). The CO₂ production is estimated by dividing the infusion rate of ¹⁴CO₂ by the concentration of ¹⁴CO₂ excreted. This technique was originally developed to determine the specific radioactivity of ¹⁴CO₂ in the blood (Young et al., 1969) or urine (Havstad and Malechek, 1982), but later saliva withdrawn from the parotid gland using peristaltic pumps were employed (Sahlu et al., 1988; Sanchez and Morris, 1984). Limitations also exist for this technique (Lawrence et al., 1991; Whitelaw, 1974), including the use of a radioactive element (¹⁴C) that has led to the modification of the technique to use its stable non-radioactive isotope (¹³C). Regardless of which isotope to use, there have been reports of overestimating CO₂ production (Havstad and Malechek, 1982; Prieto et al., 1997; Sahlu et al., 1988).
5. The δ_{PDB} ¹³C-to-¹²C ratio technique uses stable, non-radioactive C isotopes to estimate EE (Carro et al., 1980; Chevalier et al., 1984; Jones and Lefeuvre, 1989) and as a tracer in metabolism trials to study the nutrient utilization by animals (Tyrrell et al., 1984). The CO₂ is analyzed with a mass spectrometer adapted to stable isotope analysis, and the ratio of the masses of ¹³CO₂ and ¹²CO₂ in the sample is compared to the ratio of a standard gas of known ¹³CO₂-to-¹²CO₂ ratio. The isotopic composition of the sample is expressed in terms of relative difference (‰) compared to the Pee Dee Belemnite (PDB) limestone, which contains 1.111% ¹³C) standard (Carro et al., 1980). However, different forages have different natural concentrations of ¹³C that may interfere with the δ_{PDB} ¹³C-to-¹²C ratio, leading to incorrect estimates of CO₂ production.

Alternative techniques, which are calibrated with calorimetry under controlled experimentation, can determine HP through different means and provide more feasible and practical determinations of grazing animals' EE, including:

1. The heart rate technique provides a satisfactory correlation between heart rate and EE in free-ranging and wild ruminants (Chabot, 1993; Lawrence et al., 1991; White, 1993), but its main limitation lies in its very application in field animals because it requires calibration for individual animals.
2. The near-infrared (**NIR**) laser technique is a noninvasive technique based on the NIRS of oxyhemoglobin (Hb-oxy) and deoxyhemoglobin (Hb-deoxy) of tissues (e.g., brain) *in situ* according to Lambert-Beers law, which defines the relationship between concentration and infrared absorbance. Studies conducted with Cheviot sheep (Takahashi and Eda, 1997) to monitor changes in Hb-oxy and Hb-deoxy at 775, 808, 825, and 800 nm wavelengths reported metabolic rate of the whole body of 75.7 watts at resting and 132.8 postprandial (about 18 min after feeding), which corresponds to 65.1 and 114.2 kcal/h (1 watt = 0.86 kcal/h), respectively. Assuming a shrunk body weight (**SBW**) of 55 kg, the average NE requirement would be 77.4 kcal/kg^{0.75}/d. Others have also determined EE in calves using cerebral hemoglobin oxygenation (Pringle et al., 1998b; Pringle et al., 1998a).

Energy Expenditure for Grazing Animals

Grazing systems are the central system of cattle production in tropical and subtropical ecozones, whereas in temperate ecozones, grazing is explored at different seasons and intensities. As aforementioned, the EE for physical activities and forage browsing of grazing animals is primarily the only additional energy cost compared to confined animals, assuming the dietary ME is similar. Therefore, the correct understanding of EE for physical activities and quantifying the nutrient requirements for such animals under diverse conditions is necessary to develop more efficient feeding systems to optimize energy and protein use and assist with supplementation strategies (Tedeschi et al., 2019).

The ARC (1980) developed a factorial approach to estimate the EE (kcal/d) (Eq. [3]) associated with physical activities by assigning coefficients to the number of hours animals spent standing (h/day), the number of body changes (laying down and standing), and horizontal and vertical (ascent) locomotion (km/day). Appendix 1 has the relationships between these locomotion calculations. Assuming the typical values for feedlot and continuous grazing of 12 and 18 h/d, 6 times/d, 0 and 2 km/d, and 0 km/d; respectively, the EE values for animals' physical activity with 300 kg of body weight (**BW**) are 471.6 and 1024.6 kcal/d, respectively. If the required NE for maintenance is assumed to be 70 kcal/kg^{0.75} BW/d, these EE values for physical activity become an additional 9.35 and 20.3%, respectively. That means, the daily required NE for maintenance becomes $1.0935 \times (70 \times \text{BW}^{0.75})$ and $1.203 \times (70 \times \text{BW}^{0.75})$ for animals under feedlot and continuous grazing conditions, respectively.

$$\text{Physical Activity} = (0.1 \times \text{Standing} + 0.062 \times \text{Position Changes} + 0.621 \times \text{Horizontal Locomotion} + 6.69 \times \text{Ascent Locomotion}) \times \text{BW} \quad [3]$$

The CSIRO (2007) devised a different approach to compute the EE of grazing animals by adding an assessment of animals' DMI and grazing density (animals per ha), and dry matter digestibility (**DMD**) and availability of the forage to the animals' physical activity (horizontal locomotion). While this is a more mechanistic approach to estimate EE of grazing animals, it requires additional information that might be neither available nor measurable. Novel methods and techniques to assess forage availability and quality (González et al., 2018) are promising, and their adoption in these situations will help our understanding of grazing animals' EE tremendously.

Table 1 has a revised literature data published by Tedeschi and Fox (2020a), which was initially reported by Tedeschi (2001) and Tedeschi and Fox (2015), who provided a detailed description of the studies. The dataset summarizes EE for different grazing ruminant species, using different techniques from 18 studies, and a summary of Israeli studies for grazing Simmental x Hereford beef cows, using the heart rate technique and global positioning system from four studies. Studies are listed in Table 1, and Figure 1 has the boxplot of EEs for basal metabolism, chewing (i.e., eating, ruminating), and locomotion (horizontal, vertical, and ascent). Assuming an animal walking 1 km/d either on the horizontal, vertical, or ascent, Figure 1 provides a relative comparison of EE (kcal/kg^{0.75} BW) for these activities on a daily basis. After the basal metabolism, eating would have the second-largest EE for grazing animals, which is likely to hold for confined animals.

The average values for horizontal and vertical locomotion are nearly identical between the two datasets (Table 1), but the horizontal locomotion differs considerably from the values

recommended by ARC (1980) and CSIRO (1990). For instance, for the average values of both datasets versus the ARC (1980) and CSIRO (1990) values, a 300-kg cow would require about 33 (300×0.11 kcal/kg BW/km) versus 186 (300×0.62 kcal/kg BW/km) for horizontal distance walked. The EE values for standing are very similar.

Table 1. Summary of additional energy expenditure for diverse physical activities ⁽¹⁾

Items	Standing, per day	Walking, per day	Grazing, per day	Walked distance		
				Horizontal, per kilometer	Vertical, per kilometer	Ascent, per kilometer
Israeli's data ⁽²⁾						
Kcal/kg ^{0.75} BW						
Average	11.7	19.7	22.4	0.51	6.19	54.4 ⁽⁴⁾
SD	1.17	2.76	1.18	0.16	1.34	—
CV, %	10	14.0	5.3	31.5	21.6	—
Kcal/kg BW						
Average	2.59	4.37	5	0.11	1.37	12.1 ⁽⁴⁾
SD	0.22	0.59	0.44	0.03	0.23	—
CV, %	8.4	13.6	8.8	28.1	16.9	—
Tedeschi (2001) ⁽³⁾						
Kcal/kg ^{0.75} BW						
Average	—	—	—	0.46	5.16	24.8
SD	—	—	—	0.13	1.83	2.46
CV, %	—	—	—	28.8	35.4	19.8
Kcal/kg BW						
Average	—	—	—	0.11	1.33	6.59
SD	—	—	—	0.03	0.11	0.78
CV, %	—	—	—	29.3	16.2	11.9
ARC (1980)						
Kcal/kg BW	2.39	—	—	0.62	1.32 ⁽⁴⁾	6.69

⁽¹⁾ Revised from Tedeschi and Fox (2020a).

⁽²⁾ References include 21: Aharoni et al. (2009); 22: Aharoni et al. (2013); 19: Brosh et al. (2006); 20: Brosh et al. (2010).

⁽³⁾ References include 2: Corbett et al. (1969); 3: Di Marco and Aello (1998); 4: Dijkman and Lawrence (1997); 5: Fall et al. (1997); 6: Havstad and Malechek (1982); 7: Herselman et al. (1998); 8: Lawrence and Richards (1980); 9: Lawrence and Stibbards (1990); 10: Méndez et al. (1996); 11: Nienaber et al. (1993); 12: Prieto et al. (1997); 13: Ribeiro et al. (1977); 14: Sahlu et al. (1988); 15: Sanchez and Morris (1984); 1 and 16: Susenbeth et al. (1998); 17: Thomas and Pearson (1986).

⁽⁴⁾ Ascent EE = [Vertical EE – Horizontal EE $\times \cos(6^\circ)$]/ $\sin(6^\circ)$, assuming a 6° angle. See Appendix 1.

For goats, the EE for horizontal walking varied from 0.8 to 0.87 kcal/kg BW/km, for vertical ascent walking was 7.58 kcal/kg BW/km, for eating was 0.43 kcal/kg BW/kg of dry matter (**DM**) for pelleted food and grain concentrates and from 1.55 to 2.87 kcal/kg BW/kg DM for chopped and long dried forages, respectively (Lachica and Aguilera, 2005). The EE for horizontal walking and vertical ascent walking was similar to that recommended by the ARC (1980) and CSIRO (1990), but seven times greater than that reported in Table 1 by Tedeschi (2001). Amazingly, the cost of eating (kcal/kg BW/kg DM) in goats was at least 8 and 1.4 times more expensive than that reported for cattle and sheep, respectively, consuming similar diets (Lachica and Aguilera, 2005).

The main disadvantage of different techniques to assess EE of grazing animals is that their results vary tremendously (Figure 1), but this is partially explained by the variations in feed quality and amount used and types of animals under different environments in each experiment. Thus, a direct comparison of the results is complicated.

Tedeschi and Fox (2015) and Tedeschi and Fox (2020a) proposed a holistic approach to predict the increase in the NE required for maintenance due to physical activities, eating, and ruminating forages of different quality based on **1)** the energy partitioning proposed by the NRC (1981), **2)** the basal metabolism proposed by Lofgreen and Garrett (1968) adjusted for physical activity proposed by Fox and Tylutki (1998), **3)** Eq. [3] to estimate physical activity, and **4)** the data reported by Susenbeth et al. (1997, 1998) to estimate the EE for chewing (i.e., eating and ruminating). Eq. [4] shows the calculation of ME required for maintenance, but because the EE for chewing requires dietary ME at maintenance to compute ME intake at maintenance, it can only be solved iteratively through optimization. Figure 2 depicts a simulation of EE and energy partitioning using Eq. [4].

$$ME_{mr} = \left[\begin{aligned} & \left((0.07 \times SBW^{0.75} - 1.9) + \right. \\ & 0.001 \times \left(\begin{aligned} & 0.1 \times \text{Standing} + 0.062 \times \text{Position Changes} + \\ & 0.621 \times \text{Horizontal Locomotion} + \\ & 6.69 \times \text{Ascent Locomotion} \end{aligned} \right) \times FBW + \\ & \left. 4.2557 \times DMI \times MEI^{-0.95878} \right] / km \end{aligned} \right] \quad [4]$$

The simulation was conducted for animals at 250 and 450 kg SBW, the horizontal locomotion varied from 1,500 to 0 meters per day, and the vertical locomotion varied from 500 to 0 meters per day for dietary ME varying from 1.5 (low-quality forage) to 3.5 (grain-based diet) Mcal/kg, respectively. The logic behind the variable horizontal and vertical locomotion versus dietary ME was that animals would have to graze farther to meet their energy needs on low-quality forages than those on high-quality forages. It was assumed that horizontal and vertical locomotion would decrease 75 and 25 m for each 0.1 increase in dietary ME. Standing and body position changes were assumed to be 12 h/d and 6 times/d, respectively.

Figure 2B shows that the proportion of MEI (i.e., ME_{mr}) that was lost (i.e., heat increment) was identical between the 250- and 450-kg animals because the partial efficiency of use of ME to NE for maintenance (k_m) was computed from dietary ME, which was identical for both animals. In reality, both k_m and k_g could change differently between these animals if the body composition was considered (Tedeschi et al., 2004). The heat increment varied from 1.84 to 9.16 Mcal/d for the 250-kg animal and 3.1 to 13.9 Mcal/d for the 450-kg animal. However, the proportion of MEI used for chewing was more remarkable for the 250-kg animal (19% to 22%) than for the 450-kg animal (13% to 14%). Similarly, the EE for chewing was more significant for low-quality forage (1.5 Mcal/kg) than grain-based finishing diets (3.5 Mcal/kg DM). The proportion of physical activity (i.e., movement or locomotion) was similar, ranging between 7% and 14%.

Based on this simulation, animals consuming diets containing 1.5 to 1.9 Mcal ME/kg DM (or less) will likely not meet their daily energy needs and may lose BW. In fact, the 1.9 Mcal ME/kg DM

is approximately 53% TDN, which is similar to the 50% suggested by Van Soest (1994; Figures 7.8 and 7.9) for cattle to maintain their energy balance.

Challenges in Determining Voluntary Feed Intake

Although the utilization of technologies in animal nutrition has improved significantly in the last decades, the need for reliable prediction methods to estimate voluntary feed intake (**VFI**) by grazing ruminants still limits a broader application of nutrition models (Galyean, 2020). The utilization of empirical equations adopted by the NRC (2016; 2000) and NASEM (2016) has shown flaws when predicting DMI for grazing animals (Coleman et al., 2014; Lardy et al., 2004). The primary issue with utilizing empirical equations is that the accuracy pre-established might only be achieved when utilized under similar settings. The data utilized to develop the equations originated, which accentuates the problem that pen setting studies will rarely reflect grazing behavior. Predictors such as NDF (Mertens, 1987) and NEm concentration in the feed (NRC, 2016; NASEM, 2000) are somewhat utilized when predicting DMI, which strongly relies on the physical and metabolic mechanisms that seem to control VFI (Poppi et al., 1989).

Even though the physical mechanisms might fit well as a regulatory means for grazing animals while the metabolic or the chemostatic seems to be more applicable in feedlot conditions, mechanistic models that integrate both approaches appear to be more reasonable in explaining variation in VFI (Detmann et al., 2014). Tedeschi and Fox (2020a) illustrated the variability of VFI in two different grazing scenarios, poor-quality forage and a good-quality forage (40% *in vitro* DMD, 40% NDF, and 9% CP versus 75% *in vitro* DMD, 65% NDF, and 10% CP, respectively) based on the mechanistic model proposed by Fisher (1996), whose model adopted both physical distention and chemostatic effects to estimate VFI simultaneously. The DMI estimated were 1.6% and 2.4% of BW for the poor- and good-quality forages, respectively. In addition to the lower intake for the poor-quality forage, the model suggests that animals consuming poor-quality forages would have more significant daily variation in the intake than animals consuming good-quality forages. The NASEM (2016) suggested that forage availability and quality must be incorporated in prediction models to estimate VFI for grazing animals. Several studies have shown that ADG and feed intake are more associated with green pasture (i.e., quality; OM available/kg BW) than total forage mass per se (kg DM/ ha) (Minson, 1990).

The use of new technologies to estimate forage availability has been extensively studied in the last few years (Reinermann et al., 2020; Wigley et al., 2019; Woodward et al., 2019), its applicability in real scenarios is still limited (Kallenbach et al., 2020). The influence of forage availability in the eating behavior alters animal activity (Minson, 1990); thus, increasing animals' NEm requirements (Fox and Tylutki, 1998). A better understanding of forage intake for grazing ruminants seems pivotal to comprehend grazing ruminants' behavior and how forage quality affects animal activity, contributing to a better EE account and improving energy requirement estimation.

Conclusions

The EE for physical activity and chewing accounts for nearly all the differences between confined and grazing ruminants, and yet, our incomplete understanding of these components keeps

rebounding time after time without a more definitive solution. In part, data collection of plant and animal interaction (forage selection, grazing behavior, pasture growth/regrowth, pasture quality, nutrient digestion and absorption, volatile fatty acids production and profile, energy requirement) remains a critical bottleneck for adequate knowledge of forage intake by ruminants (Tedeschi et al., 2019). The majority of the data on EE for ruminants climaxed in the early 1960s towards the mid-1980s, boosted by many open-circuit, indirect calorimetry apparatuses and methods, but none can be tagged as ideal as limitations exist. New data is needed. The scientific community has been under a spell since then, and only recently, in the last five years, we have been invigorated by the advance of sensors (and artificial intelligence) that has allowed us to embark on a gradual crescendo of excitement. We must continue to boost the investment in noninvasive techniques onwards; otherwise, we will once again paralyze our understanding of energy needs by grazing animals, jeopardizing our search for sustainable livestock production, and upsurge our dependability on feedlots to produce meat to satisfy the population demand.

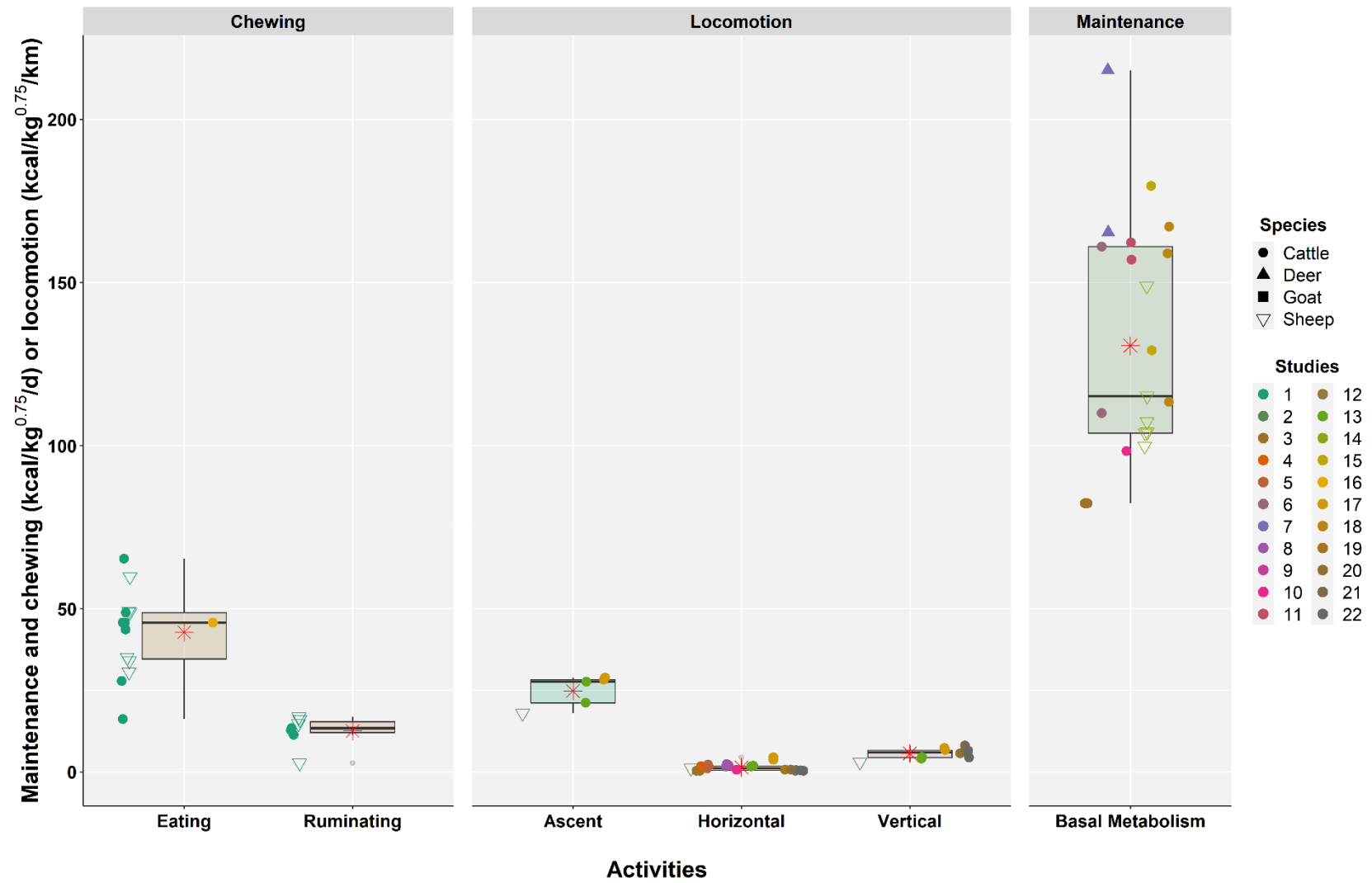


Figure 1. Boxplot of energy expenditure for chewing and locomotion activities using the datasets described in Table 1. The red asterisks indicate the average.

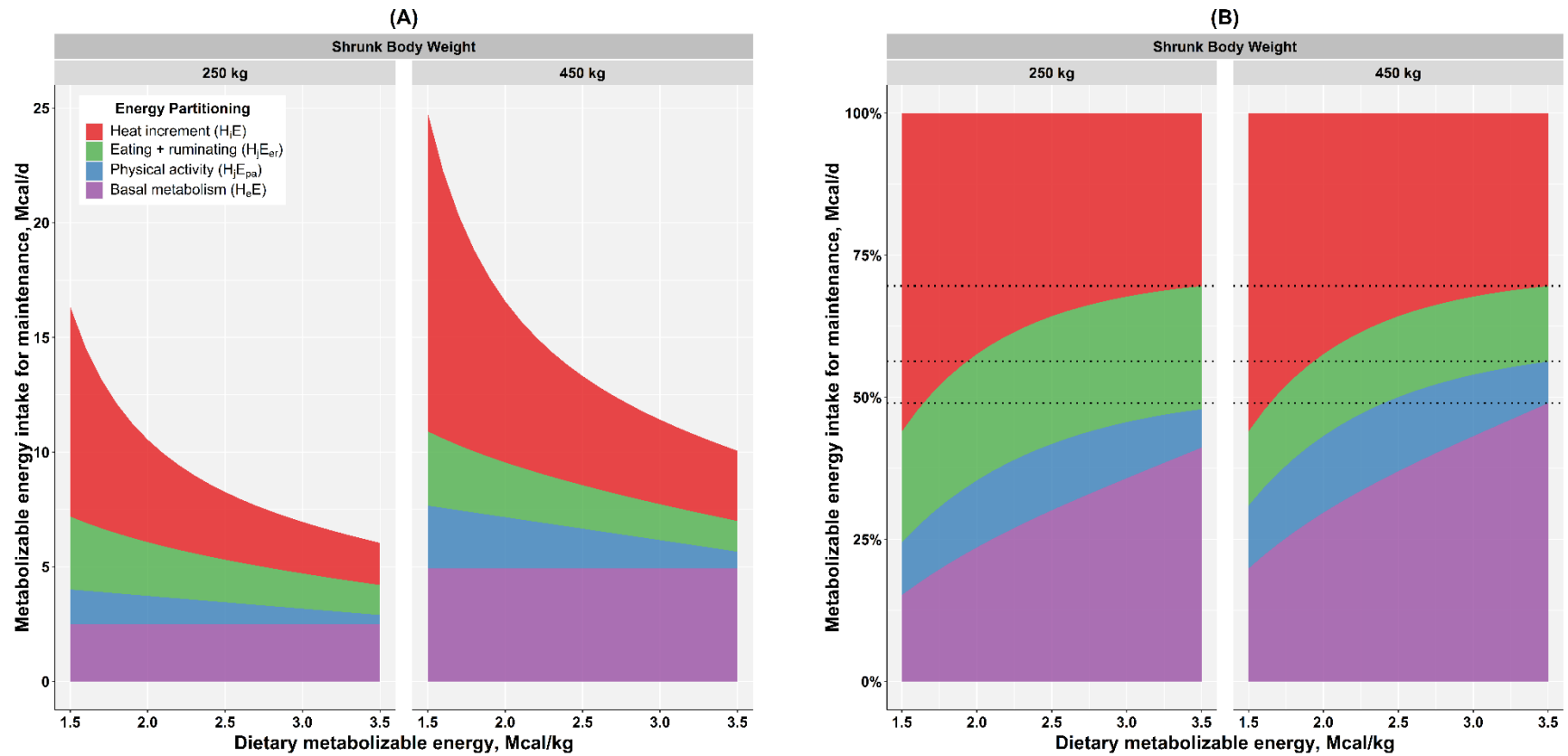


Figure 2. Amount (A; Mcal/d) and relative proportion (B; percentage) of predicted energy required for basal metabolism (H_eE), physical activity (H_jE_{pa}), eating and ruminating (H_jE_{er}), and heat increment (H_iE) for animals at 250 and 450 kg of SBW fed diets containing from 1.5 to 3.5 Mcal of ME/kg of DM at maintenance-level intake. It was modified from Tedeschi and Fox (2020a) with permission.

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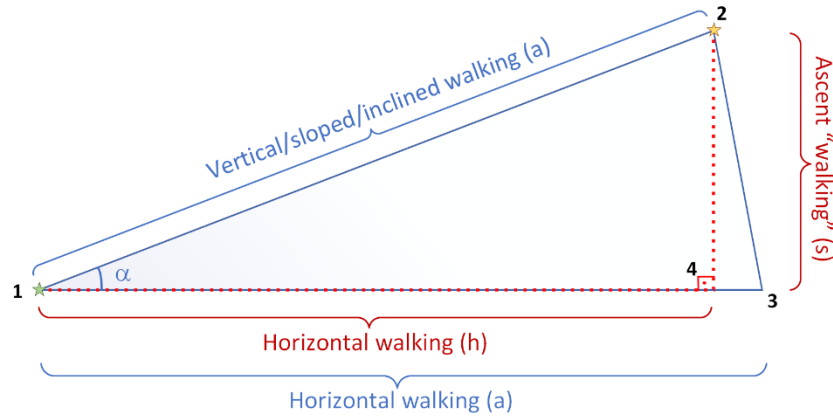
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Appendix 1. Relationship between energy expenditure for horizontal, vertical, and ascent locomotion.

In practice, an animal's locomotion is either on leveled terrain (horizontal, flat) or sloped terrain (vertical) with a gradient or an inclination angle (α). The main limitation of measuring the energy expenditure (EE) for sloped terrains is that the inclination angle may differ between studies, making it challenging to compare measured EE needed for vertical locomotions. Thus, the EE of ascent locomotion removes the inclination angle as it assumes the differences in heights only to estimate EE.



Thus, as shown in the Figure above, an animal can go from point 1 (green star) to point 2 (yellow star) by directly walking vertically from $1 \rightarrow 2$, or walking horizontally from $1 \rightarrow 4$ and then ascent from $4 \rightarrow 2$. Given the laws of thermodynamics of conservation of energy, it is assumed that the $a \times EE_{1 \rightarrow 2}$ is equal to the $h \times EE_{1 \rightarrow 4}$ plus $s \times EE_{4 \rightarrow 2}$, assuming that the EE is expressed as energy per distance unit, and a , h , and s are distances. Hence, we have:

$$EE_{1 \rightarrow 2} \times a = EE_{1 \rightarrow 4} \times h + EE_{4 \rightarrow 2} \times s$$

The h and s can be computed from the triangle 1-2-4 using trigonometric relationships, as follows:

$$\cos(\alpha) = h / a \therefore h = \cos(\alpha) \times a$$

$$\sin(\alpha) = s / a \therefore s = \sin(\alpha) \times a$$

Replacing h and s , simplifying the equation, and re-arranging to estimate $EE_{4 \rightarrow 2}$, we have:

$$EE_{1 \rightarrow 2} \times a = EE_{1 \rightarrow 4} \times \cos(\alpha) \times a + EE_{4 \rightarrow 2} \times \sin(\alpha) \times a \therefore EE_{4 \rightarrow 2} = (EE_{1 \rightarrow 2} - EE_{1 \rightarrow 4} \times \cos(\alpha)) / \sin(\alpha)$$

Because $EE_{1 \rightarrow 4}$ is the same as $EE_{1 \rightarrow 3}$ as they are expressed as energy per distance, we can substitute $EE_{1 \rightarrow 4}$ with $EE_{1 \rightarrow 3}$ to obtain the final equation to compute $EE_{4 \rightarrow 2}$ with known measurements expressed as energy per distance. Note that $EE_{4 \rightarrow 2}$ may not be the same as $EE_{2 \rightarrow 4}$.

$$EE_{4 \rightarrow 2} = (EE_{1 \rightarrow 2} - EE_{1 \rightarrow 3} \times \cos(\alpha)) / \sin(\alpha)$$



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Investigating Behavioural and Brain Responses as well as Gut-Brain Signals to Understand the Relationship Between Nutrition and Health in the Pig Model

Exploration des réponses comportementales et cérébrales et des signaux intestin-cerveau pour comprendre la relation entre nutrition et santé chez le modèle porcin

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Abstract

Pigs are increasingly used for research at the intersection of nutrition and health. In addition to be farmed principally for human nutrition, they are also used as animal models in the scope of biomedical research. Here we will mainly focus on animal nutrition to demonstrate whether the type of diet, nutritional stimuli, and even functional food ingredients can modulate the pig's behaviour, welfare, and brain functioning. Even though exploratory methods combining behavioural tests and *in vivo* brain imaging cannot be widely used in pig husbandry, they can be implemented in the scope of R&D strategy to provide valuable information about the effects of food and diet on animals' welfare and health. Complementary approaches targeting the gut-brain axis are usually used to understand the mechanisms of such a relationship, for example to relate neurobehavioural criteria with the gut microbiota, hormonal regulation, or immunity and inflammation processes. Several behavioural tests (*e.g.* food preference and motivation tests, openfield, holeboard, mazes, novelty-suppressed feeding, *etc.*) and brain imaging modalities (*e.g.* nuclear imaging, functional magnetic resonance imaging) will be presented to highlight their respective interests, pros and cons. Various instances of published research will then illustrate how these exploratory strategies can be implemented in the context of eating behaviour disturbances (exacerbated or insufficient appetite) or psychosocial stress for example. A focus will be done on the use of functional food ingredients in pig nutrition to improve food pleasure and adaptation to stressful conditions.

Résumé

Les porcs sont de plus en plus utilisés pour la recherche à l'intersection de la nutrition et de la santé. En plus d'être élevés principalement pour l'alimentation humaine, ils sont également utilisés comme modèles d'étude dans le cadre de la recherche biomédicale. Ici, nous nous intéresserons principalement à la nutrition animale pour mettre en évidence la manière dont le type de régime, les stimuli nutritionnels et même les ingrédients alimentaires fonctionnels peuvent moduler le comportement, le bien-être et le fonctionnement cérébral chez le porc. Même si les méthodes exploratoires combinant tests comportementaux et imagerie cérébrale *in vivo* ne

peuvent pas être largement utilisées en élevage porcin, elles représentent un atout dans le cadre de stratégies R&D pour fournir des informations précieuses quant aux effets de l'alimentation et du régime sur le bien-être et la santé des animaux. Des approches complémentaires ciblées sur l'axe intestin-cerveau sont généralement utilisées pour comprendre les mécanismes d'une telle relation, par exemple pour relier les critères neurocomportementaux au microbiote intestinal, aux processus de régulation hormonale, d'immunité et d'inflammation. Plusieurs tests comportementaux (*e.g.* tests de préférence et de motivation alimentaires, *openfield*, *holeboard*, labyrinthe, alimentation supprimée par la nouveauté, *etc.*) et modalités d'imagerie cérébrale (*e.g.* imagerie nucléaire, imagerie par résonance magnétique fonctionnelle) seront présentés pour mettre en évidence leurs intérêts respectifs, leurs avantages et inconvénients. Différents exemples de recherches publiées illustreront ensuite comment ces stratégies exploratoires peuvent être mises en œuvre dans le contexte de troubles du comportement alimentaire (appétit exacerbé ou insuffisant) ou de stress psychosocial par exemple. Un accent sera mis sur l'utilisation d'ingrédients alimentaires fonctionnels en nutrition porcine pour améliorer le plaisir alimentaire et l'adaptation aux conditions stressantes.

Introduction

Numerous reviews have been written to describe the specificities and advantages of the pig model in studies focusing on behavior, nutrition and neuroscience (Kornum and Knudsen, 2011, Roura *et al.*, 2016, Val-Laillet, 2019). Besides the fact that the pig is an animal species of obvious interest in agriculture and human food production, it also has incomparable advantages when it comes to studying the consequences of the nutritional environments, diets or feed in itself on animal health, and allow its extrapolation to human health. Certain questions raised in animal production find an echo in the field of human nutrition and the problems inherent in loss of pleasure and appetite linked to changes in the environment or stressful situations, or in digestive and nutritional disorders induced by suboptimal/deleterious diets. The issue of excessive weight gain also exists, and although it affects pets more than production animals, it represents an undeniable parallel with the obesity pandemic described in human populations. This comes within the scope of the “One Health” concept.

This short paper is not a literature review but a quick panorama of the author's teamwork in the context of neurobehavioral research in pigs. Here, instead of discussing about translational science, we will rather provide a quick overview on the exploratory methods that can be used in pigs to investigate the impact of food and diets on behavior and welfare, as well as the technologies that can be used to illustrate their brain correlates. These strategies can be combined with laboratory approaches aimed at investigating the gut microbiota, hormonal regulation, or immunity and inflammation processes to provide a picture of how the microbiota-gut-brain axis is impacted by nutrition. Of course, neurobehavioral approaches are often time-consuming and require complex paradigms and/or access to high-technology imaging platforms. Even though exploratory methods combining behavioural tests and *in vivo* brain imaging cannot be widely used in pig husbandry, they can be implemented in the scope of research and development strategies to provide valuable information about the effects of food and diet on animals' welfare and health.

Investigating the effects of food and nutrition on pigs' behavior

The main goal of nutrition is to provide calories and nutrients to feed the organism, maintain its basal metabolism, and ensure its growth and reproduction. But beyond these physiological and metabolic requirements necessary for survival, good health and perpetuation of species, food also contributes to the individuals' hedonic needs and welfare. The nature of diet and food items available can modulate the animals' behavior and activity, its cognition, the way it learns and memorizes things, the way it behaves with social partners and humans, the way it reacts and adapts to its environment. Since animal production environments are far from natural living conditions and mostly not optimal in terms of animal welfare and cognitive development, food can represent a vector to help the animals coping with their breeding conditions. On the contrary, when the nutritional environment is not adapted, it can have deleterious consequences on the animals' development and welfare, contributing to cognitive, emotional, and health problems. To picture the impact of nutrition on pigs' behavior, it is necessary to explore the different dimensions of animal behavior, and identify which factors deserve attention and possibly corrective interventions. Many behavioral tests have already been implemented in pigs to this purpose (**Figure 1**).

Eating behavior of course represents a major question of interest for animal productions. Feed must fulfill the animals' nutritional requirements but also its hedonic expectations, which explains why so many feed producers work on feed palatability (the hedonic reward) to increase its appetite (its translation in terms of consumption). Eating behavior in itself depends on different cognitive components, including liking, wanting, and learning. In humans it is quite easy to disentangle these dimensions using psychological tests and declarative questionnaires. In pigs and animals in general, this work can be achieved *via* specific experimental paradigms and apparatuses. Individual feed preferences (*i.e.* liking) can be explored *via* multiple-choice feed tests (*e.g.* using multi-compartment troughs, T- or Y-maze tests) and illustrated thanks to different criteria: *e.g.* visual/olfactory initial attraction conditioning the first choice, quantity consumed, time spent in contact with each feed, *etc.* We used these tests in juvenile pigs to compare different food flavors or feed additives (Clouard *et al.*, 2012b), or to investigate the preferences for sweet and/or fatty foods in minipigs (Gautier *et al.*, 2020), showing the existence of individual preferences towards specific flavours, tastes or textures. Meal microstructure (number and size of bites, interbites pauses, meal duration and intermeal periods, ingestion speed over time, *etc.*) can be investigated with computer-controlled automated feeders and provide additional data, notably to characterize satiation (short-term regulation signals) and satiety (long-term regulation signals) indicators (Val-Laillet *et al.*, 2010, Boubaker *et al.*, 2012, Clouard *et al.*, 2014a), which is very interesting to evaluate the satiating effects of feed formulations. Food-rewarded operant conditioning is a potent strategy to investigate the “wanting” dimension of eating, since the quantity of work required to obtain a feed reward can be manipulated. In the progressive ratio task, the subject is required to make an increasing number of operant responses (*e.g.* to push a button or lever) for each successive reward, which is an indicator of food motivation (Ochoa *et al.*, 2014). Different feed formulations can be compared using this approach to document the animals' willingness to work for them.

In addition but also in relation to eating behavior, the emotional, cognitive and social dimensions of behavior can be explored *via* dedicated tests. To document the emotional reactivity of pigs,

classical procedures such as the openfield test are widely used, where behavioral items are recorded in animals isolated (or not) in a novel environment, usually a square arena divided into virtual zones (Donald *et al.*, 2011, Val-Laillet *et al.*, 2013, Menneson *et al.*, 2019). Locomotor activity, vocalisations, exploratory behavior, miction/defecation, escape attempts are part of the usual behavioral repertoire studied. Similar arenas can be used to assess the emotional reactivity to known or unknown humans, familiar or novel objects, or sudden stimuli (*e.g.* noise, light, falling object, *etc.*). All these tests are potent methodological tools to investigate the animals' emotional reactivity to familiarity, novelty, suddenness, and unpredictable environments. They are valuable to describe the individual behavioral traits (*i.e.* habitual behavioral patterns stable across situations and time and providing a foundation for predicting behavior). Conflicts of motivation are sometimes very interesting to look at in order to identify individual valuation of resources and motivation priorities in pigs. This is the case for the novelty-suppressed feeding test (NSF): the animal is facing a trough containing a palatable feed but a novel and possibly frightening object stands alongside or above it (Menneson *et al.*, 2019). The pig consequently has to fight against its fear to fulfil its motivation to eat. Contention tests also rely on such a conflict of motivation (*i.e.* "freeze, fight or flight"). In piglets, tonic immobility is correlated with emotional reactivity and stress/resignation susceptibility, similar to contention tests in the adult age (*e.g.* in a hammoc or Pavlov stand) (Menneson *et al.*, 2019).

Finally, other kinds of tests can be designed to investigate the cognitive abilities of pigs, including social cognition (*e.g.* social discrimination and preferences using T- or Y-maze tests) (Val-Laillet *et al.*, 2013), but also spatial learning and memorization for example (a paragon of hippocampal-dependent tasks). The holeboard test initially designed for rodents was successfully implemented in pig models, where the animal has to memorize and retrieve palatable food rewards hidden in a testing arena, independently from olfactory cues and on the basis of spatial cues only (Gieling *et al.*, 2012, Gautier *et al.*, 2018, Gautier *et al.*, 2020). Using this paradigm, we demonstrated the deleterious effects of perinatal or adult exposure to high-sugar and high-fat diets, independently of weight gain. Every behavioural test has pros and cons as well as experimental constraints. In the case of the holeboard test, results are influenced by the cognitive abilities of the pig, *i.e.* its learning and memory capacities, but also by its motivation for the food rewards. When interpreting such tests, it is important to identify which behavioural dimensions are involved in the task, and how they can influence its outcomes. To disentangle these interconnections between different behavioral dimensions, it is possible to use different or complementary tests. For spatial learning and memorization for example, the holeboard task can be replaced by or completed with other different spatial tasks that are not reinforced by food reward, *i.e.* maze test where the success is rewarded by meeting with social partners or provision of toys instead of food (Kornum and Knudsen, 2011, Gautier *et al.*, 2018).

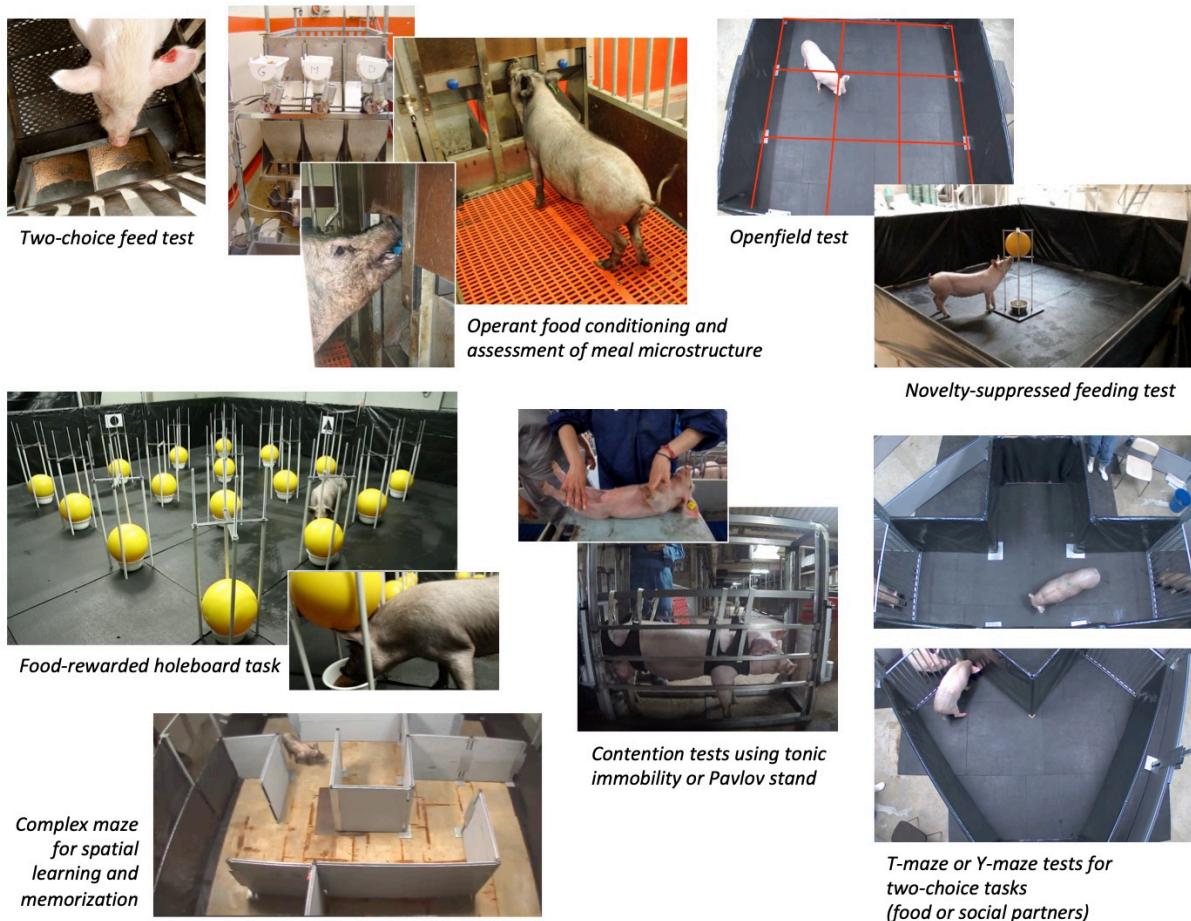


Figure 1. Examples of behavioral tests implemented in pig models (conventional or miniature pigs) to investigate food preferences, motivation, and meal structure, emotional reactivity, responses to novelty or suddenness, spatial learning and memory, or social cognition.

Investigating the microbiota-gut-brain axis in pigs

Our research department at Inrae is renowned for its expertise in functional brain imaging in pig models. For more than a decade now, we have implemented minimally invasive *in vivo* brain imaging for pigs in the context of nutrition research to investigate the impact of food and diet on brain metabolism as well as on the neurocognitive processes underlying eating behavior, with significant connections with welfare and health. This work was quite recently summarized in a review paper (Val-Laillet, 2019). **Figure 2** illustrates the kind of food stimulation or treatments investigated in conventional or miniature pigs, as well as the brain areas for which differences in terms of metabolism or functional responses were observed in juvenile or adult pigs. Most of this research focused on the motivational and hedonic dimensions of eating, highlighting how the different organoleptic, nutritional and physical dimensions of food, and where it is perceived (*e.g.* oral vs. visceral), can influence liking, wanting, and learning.

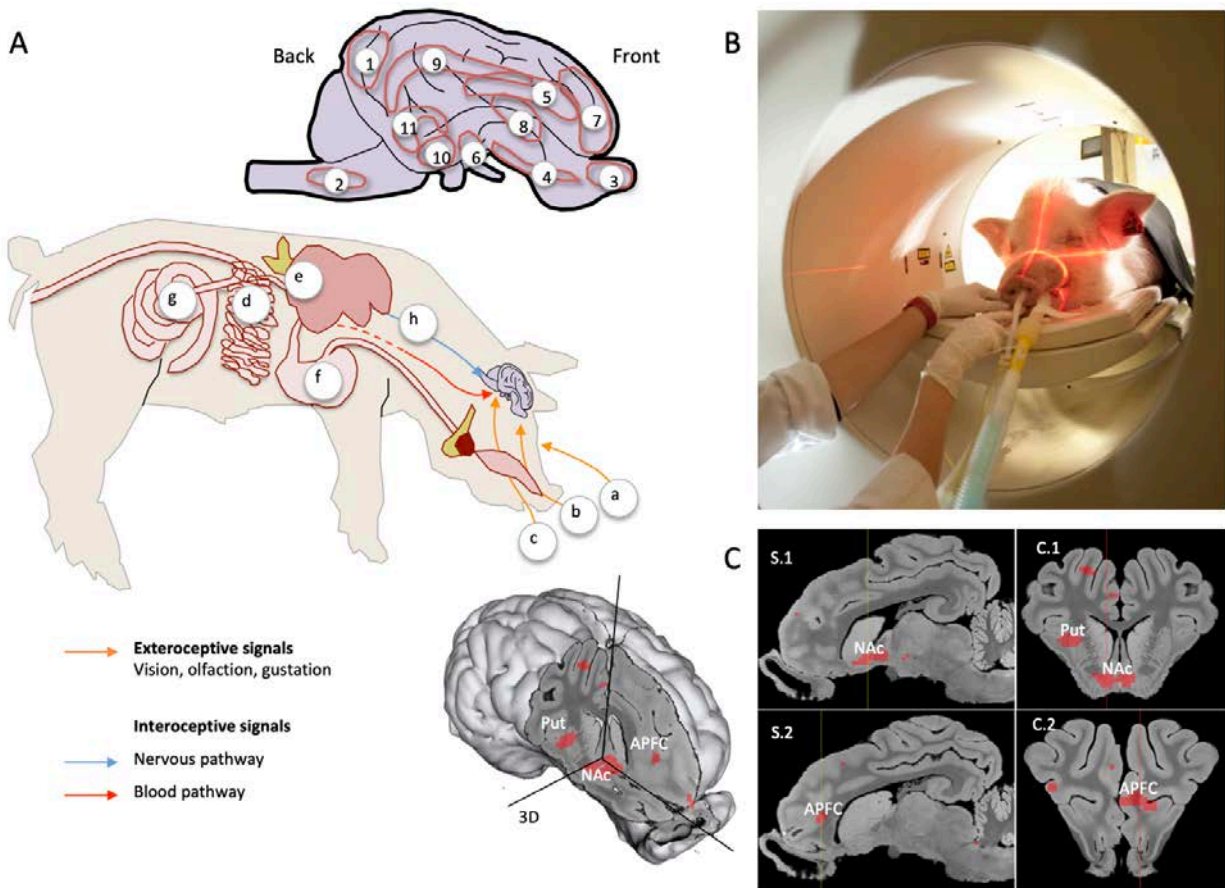


Figure 2. A) Brain responses to food stimuli, diets, and nutrition-related interventions were explored in pig models using different brain imaging modalities such as nuclear imaging (single-photo computed tomography and positron emission tomography, SPECT and PET respectively) or functional magnetic resonance imaging (fMRI). Stimulations and treatments encompassed: a) visual stimuli, b) complex odors/flavors, c) basic tastes, d) duodenal sugar infusion, e) portal sugar infusion, f) gastric distension, g) butyrate supplementation, h) vagus nerve stimulation, but also chronic Western diets and surgical interventions such as Roux-en-Y gastric bypass. In the context of these studies, various neuronal networks were described including 1) visual occipital cortex, 2) brainstem, 3) olfactory bulb, 4) prepyriform cortex, 5) insular cortex, 6) hypothalamus, 7) prefrontal cortex, 8) striatum, 9) cingulate cortex, 10) amygdala, and 11) hippocampal areas. Excerpt from (Val-Laillet, 2019). B) Anaesthetized pig being equipped with nasal and lingual catheters to investigate its olfactogustatory perception during a brain imaging session. C) Example of sagittal (S.1 and S.2) and coronal (C.1 and C.2) brain images and 3D-reconstruction obtained in the pig model, with differences of brain metabolism observed in the nucleus accumbens (NAc), putamen (Put), and anterior prefrontal cortex (APFC). Excerpt from (Gautier *et al.*, 2018).

Most of these studies were performed in the context of obesity and chronic exposure to Western diets in young or adult minipigs, in order to document the neurocognitive anomalies emerging from deleterious nutritional environments (Val-Laillet *et al.*, 2011, Clouard *et al.*, 2016a, Clouard *et al.*, 2016b, Val-Laillet *et al.*, 2017, Gautier *et al.*, 2018, Gautier *et al.*, 2020).

Comparisons between specific macronutrients in the diets were also performed, for example between fructose, glucose and starch (Ochoa *et al.*, 2016a, Ochoa *et al.*, 2016b), or between saturated fat, omega-6 and omega-3 fatty acids (Malbert *et al.*, 2021), demonstrating that the quantity but also the quality of food is determinant for the behavioral and brain developments. Some nutrients or diets favor in the pig model as in humans the onset of nutritional pathologies, including obesity and metabolic diseases, but also eating and mood disorders. Another research thematic concerned the emergence of food preferences and aversions in juvenile commercial pigs, related to amino acids ratio, basic tastes (*e.g.* sweet), or to complex flavors as those used as/in feed additives (Gaultier *et al.*, 2011, Clouard *et al.*, 2012a, Gloaguen *et al.*, 2013, Clouard *et al.*, 2014a, Clouard *et al.*, 2014b, Coquery *et al.*, 2018). A third topic deals with the use of functional food ingredients, for which a focus will be provided in the last section of this paper. Such studies are valuable to identify the food properties that modulate food intake and eating behavior, and the use of functional brain imaging is a fantastic tool to understand the neural mechanisms underlying individual choices and motivation to eat.

Amongst the different brain imaging techniques available, two main approaches were used in pigs: nuclear brain imaging, including single photon computed tomography (SPECT) and positron emission tomography (PET), as well as functional magnetic resonance imaging (fMRI). Nuclear brain imaging techniques require the injection of a radiolabelled molecule of interest in the blood system of the subject. In our studies, we used SPECT to explore the blood brain perfusion and PET to explore the brain glucose metabolism. Both techniques are highly correlated but PET provides a better spatial resolution with sometimes a more interesting biological rationale, *i.e.* the consumption of glucose by the brain cells. They can be used to investigate basal brain metabolism or the regional brain responses to specific treatments or stimulations. But investigating different stimuli requires the repetition of imaging sessions and exposes the animals to radiation, which requires controlled housing and handling systems in respect of the nuclear authority regulations. The use of fMRI does not require ionizing radiation and relies on the BOLD (blood-oxygen-level-dependent) hemodynamic response, which changes according to the variations of oxyhemoglobin and deoxyhemoglobin in the brain tissues (*i.e.* oxygen consumption). Resting-state fMRI can be performed to investigate the resting brain functions and connectivity, while BOLD responses can be recorded in response to a wide variety of stimuli, which can be repeated many times along the same imaging session. All these techniques consequently have specific advantages and constraints, and the scientific question usually orientates the best choice of methodology.

When investigating the effects of food and diet on the individuals' welfare and health, behavioral and brain measures are usually not sufficient, especially to understand the complex mechanisms underlying this relationship. Many of our research projects consequently combine these neurobehavioral explorations with measurements at the microbiota and gut levels to understand how the nutritional environment modulates the gut-brain communication and possibly the observed neurocognitive changes. The idea is usually to produce a systemic view of the metabolic, physiological, and neurocognitive effects of nutrition. Amongst the biological criteria that are recorded are gut hormones and neuropeptides involved in the regulation of homeostasis and food intake, but also biomarkers of immunity, inflammation, nervous communication, or intestinal barrier functions. The joint application of metabolomics and transcriptomics possesses the high efficiency of identifying key metabolic pathways and functional genes modulated by

nutrition. It can be used also to investigate the metabolites produced by the gut microbiota, while metagenomics is used to represent its whole genome. Canonical correspondence analyses can be performed to delineate the complex relationships between sets of variables (*e.g.* environmental and nutritional factors, physiological and metabolic parameters from the host, gut microbiota composition and activity, *etc.*) by searching for latent (hidden) gradients that associate these sets of variables.

Such a systemic exploration strategy has already been implemented in adult minipigs to document the consequences of chronic exposure to Western diets (Val-Laillet *et al.*, 2017, Gautier *et al.*, 2018, Gautier *et al.*, 2020), but also in commercial pigs to describe the microbiota-gut-brain axis and behavioral anomalies induced by chronic psychosocial stress for example (Menneson *et al.*, 2019). In minipigs, we demonstrated that the chronic consumption of Western diet induced addiction-like behavioral and brain responses to sucrose, while obesity triggered anxiety-like, snacking-type behaviors, and negative metabolic outcomes. Overall, body weight gain and loss both modulated the corticostriatal neurocognitive responses to food. In commercial pigs, we showed that social isolation and stressing unpredictable environment induced systemic deleterious consequences at the microbiota-gut-brain and behavior levels (see next section). Further analyses will be performed to relate all these effects with specific gut microbiota populations and metabolites, with the aim to design potential interventional treatments targeting the gut microbiota. For example, in a study performed in juvenile commercial pigs, we demonstrated that supplementation with butyrate, which is a microbiota metabolite produced from the fermentation of dietary fibers, impacted brain activity in regions involved in cognition and pleasure (Val-Laillet *et al.*, 2018b). A better understanding of the microbiome-host crosstalk *via* the microbiota-gut-brain axis cannot only shed light on healthy and efficient pig production but also promote our knowledge on this fascinating research topic (Wang *et al.*, 2020).

Focus: The use of functional food ingredients to improve food pleasure and adaptation to stress

Functional food ingredients or sensory feed additives are increasingly used in animal nutrition to stimulate appetite, facilitate feed transitions, or improve animal welfare. Stressful environments or events are known to have a negative impact on well-being of course, but also on eating behavior, which sometimes leads to appetite loss and anorexia, which has been well described in pigs (Clouard *et al.*, 2012a), or on the contrary to food cravings and hyperphagia. In a set of studies performed in the juvenile pig, we demonstrated positive effects of sensory feed additives or functional ingredients based on natural plant extracts and aromatic molecules on eating behavior, with increased feed preference and intake after a feed transition for example (Clouard *et al.*, 2012b, Clouard and Val-Laillet, 2014). We also showed that sensory feed additives provided to sows during gestation/lactation and/or to piglets during the post-weaning period differentially impacted animals' growth and feed intake, which highlighted the existence of nutritional programming and/or sensory conditioning during the perinatal period (Val-Laillet *et al.*, 2018a).

To interpret these positive behavioral outcomes in terms of eating behavior, we explored the brain responses to one of these functional food ingredients in naïve or familiarized juvenile pigs.

We showed that the perception of the feed additive in familiarized individuals induced different brain responses in regions involved in reward anticipation and/or perception processes than the familiar control feed flavor in naive animals (Val-Laillet *et al.*, 2016). Another study showed that these hedonic brain responses are dependent to the ingredient used and to its concentration (Coquery *et al.*, 2019). In addition to positive effects on feed palatability, we hypothesized that some functional ingredients might help the animals to cope with degraded or stressful environments. To test this hypothesis, we conceived a chronic psychosocial stress model in the pig, combining social isolation with a poor and unpredictable environment (Menneson *et al.*, 2019). Animals were characterized by a resignation behavior, altered hippocampal neuroplasticity, lower brain responses to novel food odors, higher insulin resistance and lower gut microbiota fermentation activity, revealing systemic stress-induced anomalies at the microbiota-gut-brain axis and behavioral levels (Menneson *et al.*, 2019).

Using a spice functional food ingredient, we demonstrated increased expression of 5-HT_{1A}R (serotonin receptor, a neurotransmitter involved in the reward system and mood regulation) and BDNF (a neuroplasticity marker) in the hippocampus and prefrontal cortex, respectively, as well as slight anxiolytic-like effects and significant modifications of the blood perfusion (*via* SPECT) in several brain areas involved in the regulation of emotions and cognition (Menneson *et al.*, 2020b). In another study, we observed that the BOLD fMRI brain responses induced by a pharmacologically-induced acute stress were alleviated in animals previously supplemented with a food ingredient containing *Citrus sinensis* extracts (Menneson *et al.*, 2020a). These results demonstrate that functional food ingredients can be used in pigs to modulate different behavioral dimensions and to correlate these effects with functional brain responses related to food reward, positive emotions and stress adaptation. This work opens the way to innovative nutritional strategies with the aim to improve the pigs' welfare, cognitive development, and health.

Conclusions and perspectives

This overview provided a quick panorama of some exploratory approaches designed to investigate the effects of nutrition on the microbiota-gut-brain axis, behavior, welfare and health in pig models. Of course, most of these technologies and paradigms cannot be commonly implemented on farms because they are time-consuming, require costly machines and laboratory facilities, as well as dedicated staff. Though, all these approaches represent powerful tools in the context of research and development (R&D) strategies, to conceive better diets and innovative feed ingredients contributing to the “One Health” concept and objectives. Besides these systemic R&D approaches, complementary “easy-to-implement” methods must be developed and such aims are part of the advances in precision nutrition and modern phenotyping strategies in animal production. Recent review papers also illustrate the assets provided by mechanistic modeling and data-driven models for modern animal production systems (Ellis *et al.*, 2020). Automated feeders, biological and activity sensors, environmental sensors, cameras and other image analysis tools can provide complex data streams from which it is possible to extract descriptive and predictive information in order to adapt nutrition and breeding conditions. Correlating these data with mechanistic information provided by the approaches described in this paper shall provide important interpretative keys to understand how animals perceive and adapt to their living conditions, and how it affects their behavior, welfare, and health.

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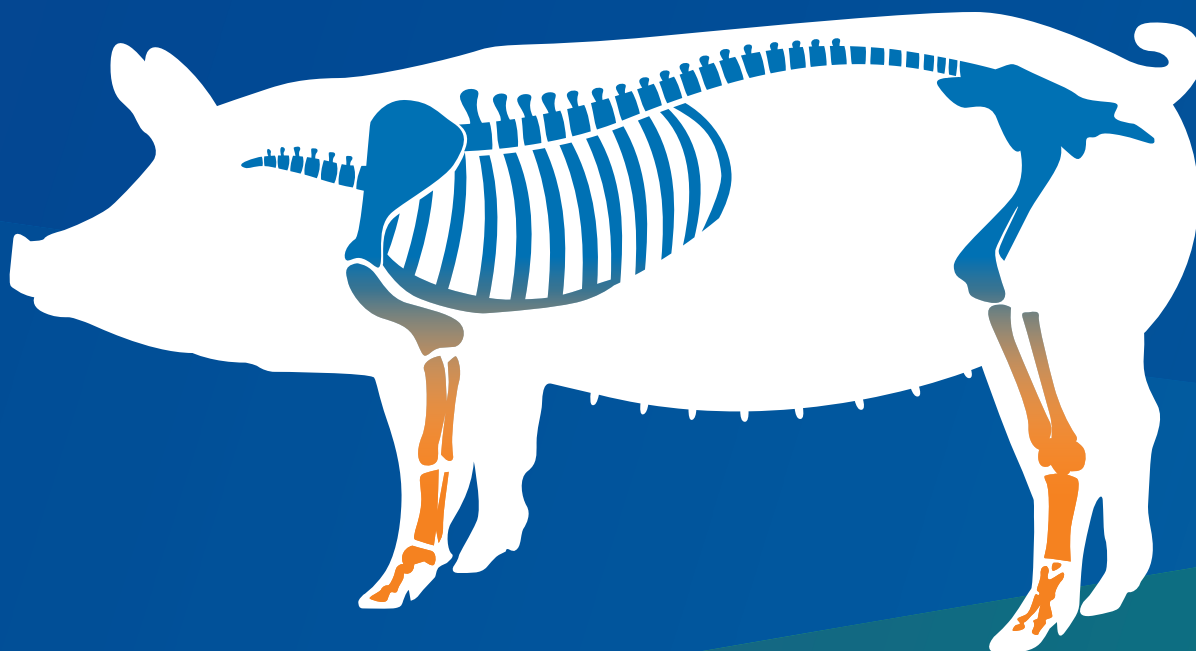
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Yeast (*Saccharomyces cerevisiae*) as a Feed Additive for Feedlot Cattle

La levure (*Saccharomyces cerevisiae*) utilisée comme additif dans l'alimentation des bovins en parc d'engraissement

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Abstract

Feed additives are derived from many origins and have been used in the animal production industry with increased use in the last three decades due to positive impacts on animal health and performance. With changing consumer demands and concerns of antimicrobial resistance, legislation limited access to in-feed antimicrobials in Canada as of December 2018. This has further increased the interest in other feed additives to improve animal growth, health, and efficiency. Yeast is a natural feed additive available in many forms, strains, and doses for livestock production; however, the majority of research in *Saccharomyces cerevisiae* yeast supplementation in livestock has focused on dairy cattle. Although modes of action have been proposed, one of particular interest for feedlot cattle is the ability to stabilize ruminal pH. It is postulated that *S. cerevisiae* aids in reducing lactate accumulation in the rumen by stimulating the production of lactic acid utilizing bacteria, ultimately increasing rumen pH (Chaucheyras-Durand et al., 2008). For feedlot cattle, the late finishing phase is that of greatest risk for ruminal acidosis and subsequent liver abscess, and poor animal performance (Nagaraja and Chengappa, 1998; Castillo-Lopez et al., 2014). Research was conducted on late finishing feedlot cattle that were supplemented with *S. cerevisiae* (60 billion colony forming units; YST) and compared to those that were not supplemented (CON) to evaluate growth performance, carcass characteristics, and indicators of rumen health (Williams et al., 2021). The results of that experiment suggested that YST decreased dry matter intake by 31% ($P < 0.001$) and improved feed conversion ($P < 0.001$) without impacting

carcass characteristics or rumen health ($P \geq 0.07$). Although this trial improved feed efficiency, the mechanisms of action of yeast for beef cattle remain unclear. Our future research program aims to increase the understanding of the modes of action of yeast as a direct-fed microbial in high-grain finishing feedlot cattle diets for the improvement of animal performance and health.

Key words: yeast, *saccharomyces cerevisiae*, feedlot, beef

Résumé

Les additifs alimentaires sont d'origines diverses et leur utilisation dans l'industrie de la production animale a augmenté au cours des trois dernières décennies en raison de leurs impacts positifs sur la santé et la performance des animaux. Compte tenu de l'évolution des demandes des consommateurs et des préoccupations liées à la résistance aux antimicrobiens, le législateur a limité l'accès aux antimicrobiens dans l'alimentation animale au Canada depuis décembre 2018. Cette situation a stimulé davantage l'intérêt pour d'autres additifs alimentaires permettant d'améliorer la croissance, la santé et l'efficacité des animaux. La levure est un additif alimentaire naturel disponible en de nombreuses formes, souches et doses pour la production du bétail; cependant, la majorité des travaux portant sur la supplémentation en levure *Saccharomyces cerevisiae* chez le bétail ont été réalisés chez les bovins laitiers. Bien que des modes d'action aient été proposés, celui qui présente un intérêt particulier pour les bovins en parc d'engraissement est l'aptitude à stabiliser le pH ruminal. On suppose que *S. cerevisiae* aide à réduire l'accumulation de lactate dans le rumen en favorisant la production de bactéries utilisant l'acide lactique, ce qui se traduit par une augmentation du pH du rumen (Chaucheyras-Durand *et al.*, 2008). Chez les bovins en parc d'engraissement, la fin de la phase de finition est celle qui présente le plus grand risque d'acidose ruminale et d'abcès hépatiques subséquents, ainsi que de mauvaises performances animales (Nagaraja et Chengappa, 1998; Castillo-Lopez *et al.*, 2014). Des recherches ont été menées sur des bovins en parc d'engraissement en fin de finition qui ont reçu *S. cerevisiae* (60 milliards d'unités formant colonie; YST) dans leur alimentation ou non (CON) afin de comparer la performance de croissance, les caractéristiques de carcasse et les indicateurs de santé du rumen (Williams *et al.*, 2021). Les résultats de cette expérience suggèrent que le traitement YST a diminué l'ingestion de matière sèche de 31 % ($P < 0,001$) et a amélioré l'indice de consommation ($P < 0,001$), sans conséquence sur les caractéristiques de carcasse ni la santé du rumen ($P \geq 0,07$). Bien que dans cet essai l'efficacité alimentaire ait été améliorée, les mécanismes d'action de la levure chez les bovins de boucherie demeurent incertains. Notre futur programme de recherche vise à améliorer la compréhension des modes d'action de la levure utilisée comme agent microbien directement dans les rations de finition riches en grain des bovins en parc d'engraissement pour améliorer les performances et la santé des animaux.

Introduction

The Canadian beef industry produces over 1.5 million tonnes of high-quality protein annually while maintaining the highest standards in animal care and environmental sustainability (CCA, 2020). In Canada, access to in-feed antibiotics has been limited to veterinary prescription and growth claims have been removed. To keep up the high standards of efficient production and prudent antimicrobial stewardship in the beef industry, alternative feed additives like yeast require

investigation to determine if they may help improve animal health and performance. Further research is required to determine if and how they can replace conventional feed additives.

Although feeding yeast to cattle is not a particularly novel concept, the majority of research has focused on dairy cattle, and responses may differ in high-grain diets. The literature indicates that yeast may help stabilize ruminal pH by reducing lactate accumulation due to high concentrate diets fed to dairy cattle (McAllister et al., 2011). The late finishing phase is the highest risk period for feedlot cattle to experience ruminal acidosis (Castillo-Lopez et al., 2014), and yeast supplementation can potentially mitigate the effects at higher dose rates during this period. Previous research in feedlot cattle indicates that supplementing yeast is promising; however, differences in yeast type and form, diet type, and dosage make comparing results across trials difficult (Buntyn et al., 2016).

An experiment was conducted to assess the impacts of supplemental *S. cerevisiae* at a high dose in the diet of late finishing cattle on animal performance, feeding behaviour, ruminal pH, ruminal volatile fatty acid concentrations, carcass characteristics, rumen wall morphology, rumen health, and biological markers of immune response and gut barrier function (Williams et al., 2021). Angus cross feedlot steers ($n = 51$) were fed a corn-based finishing ration for 55 ± 19 days with (YST) or without (CON) 60 billion colony forming units of active-dry *S. cerevisiae* yeast per day. It was observed that YST steers reduced feed intake (31%) and improved feed efficiency ($P \leq 0.001$). A reduction in dry matter intake (DMI) variation was also observed for YST steers compared to CON ($P = 0.04$). No differences were observed between treatments for average daily gain or ultrasound measurements. Previous research in beef cattle has observed an increase or no change in DMI (Beauchemin et al. 2003b; Vyas et al. 2014; Ran et al. 2018). In addition, in our experiment, we did not observe differences in circulating blood metabolites ($P \geq 0.13$); therefore, fat or protein mobilization did not appear to be the reason why YST steers, that consumed much less feed, continued to gain as much as those eating 31% more feed per day. It was speculated that the YST steers may have had improved energy efficiency; one postulated mechanism was through enhanced propionate production. However, although propionate concentration was numerically higher in these steers (YST 37.2 mol/100mol, CON 31.7 mol/100mol; $P = 0.23$), it was not significantly different. Ruminal pH and time spent in ruminal acidotic states were not different between treatments ($P \geq 0.08$) but numerically lower for YST, similarly to previous research (Mohammed et al., 2017); however, not significant ($P \geq 0.08$). This may be due to increased animal variation and limited pH sensors in the experiment. No differences were detected between treatments for carcass characteristics, rumen wall morphology, rumen health, or biological markers of immune response (TLR2, TLR4, FCAR) and gut barrier function (OCLN, CLDN, ZO1, ZO2; $P \geq 0.07$). These results were expected without differences in weight gain, ruminal kinetics, or a ruminal acidotic challenge. The results of this experiment suggest that the addition of yeast during the late finishing phase in a high-grain diet reduces dry matter intake and its variation without impacting weight gain, carcass characteristics or rumen health, thereby improving feed efficiency. *Saccharomyces cerevisiae* mechanisms of action are not well understood in beef cattle, and additional work at this higher dose range (60 billion colony forming units) using a similar yeast strain, animal, and diet type are warranted.

Proposed Research Plan

Three experiments have been developed using the same animal type (finishing beef cattle), basal diet composition (corn-based), yeast strain (*S. cerevisiae*) and dose (60 billion colony forming units). This will allow for a more thorough investigation into impacts and the mode of action of *S. cerevisiae* in high-grain beef cattle diets. Experiment 1 aims to determine the impact of the form of yeast (active-dry, heat-killed, or rumen-protected) and expected location of the activity against positive (with conventional antimicrobials) and negative (no feed additives) controls on steer performance, total tract digestibility, and ruminal pH. The second experiment will determine the impact of yeast supplementation on gut barrier function and molecular markers of gut permeability and immune response in the gastrointestinal tract of high-grain feed beef cattle. The final experiment aims to determine the impact of yeast on the microbiome of the rumen, jejunum, and colon of beef cattle fed high-grain diets with or without yeast supplementation. The overall hypothesis of this program is that the addition of yeast improves indicators of gut health and metabolic disease, helping to stabilize ruminal pH and improve overall production efficiency when fed to finishing steers. This research can offer significant benefits to the beef cattle industry by reducing metabolic disease, and improving feed efficiency and food safety through reducing livestock's contribution to antimicrobial resistance to medically important antibiotics and evaluating alternatives to antimicrobials.

This work is expected to have immediate benefits to the Canadian beef and feed industries. In addition to the potential reduction in rumen acidosis and liver abscesses, the addition of yeast may have the potential to increase feed efficiency and nutrient digestibility. Improvement in feed efficiency has four times the economic return over the same percent increase in animal performance (Gibb and McAllister, 1999). This research will increase the body of knowledge of direct-fed microbials in the feedlot sector and perhaps deliver a transformative technology to improve animal health and welfare, and improved performance efficiency for the feedlot industry.

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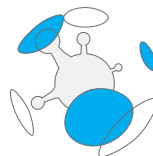
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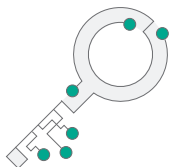
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Precision Feeding of Poultry: Matching Nutrient Supply to the Nutrient Requirements of Individual Birds

Alimentation de précision en aviculture : assurer la correspondance entre l'apport en nutriments et les besoins nutritifs individuels des oiseaux

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Abstract

The ultimate goal of every nutritionist is to perfectly match nutrients supplied with nutrient requirements of individual birds at every moment feed is consumed. The poultry industry remains a long way from realizing that goal. We must shift our thinking from flock-level to the nutritional needs of individual birds every moment of every day. We have developed a precision feeding system to feed every bird the right amount of the right feed at the right time. Uniquely, the system automates control of nutrient intake for poultry. It consists of smart feeding stations connected to a computer that communicates with every station and records the accumulated data centrally. The stations allow one bird to eat at a time, without interference from other birds. This allows us to control the formulation and level of feed intake of each bird. Research on the nutrient requirements of individuals is sparse, and models need to be built and refined to perfect the provision of the right feed to the right bird at the right time. However, even our current limited knowledge of how optimal nutrient ratios change with age, level of production, and environmental conditions can facilitate substantial improvement if we refine our current flock-level phase feeding strategies. Technology will help us shorten the time between phases. By blending diets to achieve closer real-time matches between nutrient supply and the nutrient requirements of each individual bird, we will reduce wasted nutrients and unrealized potential by an unprecedented margin. This approach will reduce FCR dramatically, while simultaneously reducing the land base required to produce feedstuffs for poultry, and the subsequent excretion of N and P, and emissions of CO₂ and NH₃. Precision feeding technology will facilitate not only the implementation of more socially responsible poultry feeding, it can also be used to conduct the research necessary to continuously improve models, and implementation of smart poultry nutrition. Big data from each individual has the potential to be leveraged by advances in machine learning and artificial intelligence. With this approach, even commercial operations will be able to participate in research with an eye to achieve every nutritionist's ultimate goal – maximum profit with minimum waste.

Résumé

Le but ultime de tout spécialiste de la nutrition animale est de faire en sorte que les éléments nutritifs fournis correspondent parfaitement aux besoins de chaque oiseau à tout moment.

L'industrie avicole est encore loin d'atteindre cet objectif. Nous devons changer notre façon de penser, c'est-à-dire abandonner l'approche troupeau pour s'intéresser aux besoins nutritifs de chaque individu à chaque instant de la journée. Nous avons mis au point un système d'alimentation de précision pour donner à chaque oiseau la bonne quantité du bon aliment au bon moment. Ce système est unique en son genre car il automatise le contrôle de l'apport nutritif aux volailles. Il se compose de stations d'alimentation intelligentes connectées à un ordinateur qui communique avec chaque station et enregistre les données cumulées de manière centralisée. Les stations permettent à un seul oiseau de manger à la fois, sans interférence des autres oiseaux. Cela nous permet de contrôler la formulation et le niveau de consommation d'aliments de chaque oiseau. Les recherches sur les besoins en nutriments des individus sont peu nombreuses, et des modèles doivent être construits et perfectionnés au point de pouvoir fournir le bon aliment au bon individu, au bon moment. Cependant, même nos connaissances actuelles limitées sur la façon dont les rapports optimaux entre nutriments varient avec l'âge, l'intensité de la production et les conditions du milieu peuvent permettre une amélioration substantielle si nous perfectionnons nos stratégies d'alimentation du troupeau par phase. La technologie nous aidera à réduire le temps entre ces phases. En mélangeant les rations pour obtenir des correspondances plus étroites en temps réel entre l'apport en nutriments et les besoins de chaque oiseau, nous diminuerons comme jamais auparavant la perte de nutriments ainsi que le potentiel non réalisé. Cette approche permettra de réduire considérablement l'indice de consommation, tout en réduisant simultanément la superficie de terrain nécessaire à la production d'aliments pour les volailles, l'excrétion consécutive de N et de P ainsi que les émissions de CO₂ et de NH₃. La technologie d'alimentation de précision facilitera non seulement l'adoption de pratiques d'alimentation plus responsables sur le plan social, mais elle peut également être utilisée pour mener les recherches nécessaires à l'amélioration continue des modèles et à la mise en œuvre de la nutrition intelligente chez les volailles. Les progrès de l'apprentissage automatique et de l'intelligence artificielle pourraient permettre d'exploiter les mégadonnées de chaque individu. Grâce à cette approche, même les exploitations commerciales pourront participer à la recherche dans le but d'atteindre l'objectif ultime de tout expert en nutrition : réaliser un profit maximum en ne générant qu'un minimum de déchets.

Introduction

Academia and industry have invested huge resources into defining both the nutrient composition of feedstuffs and nutrient requirements of meat and egg-type poultry. Both aspects are highly complex because of their dynamic nature. Nutrient availabilities depend on the genetics of each feedstuff, but also geographic region, weather, and growing conditions. These factors impact both the quantity of nutrients and the molecular structures from which those nutrients must be extracted by animals after consuming the feed (reviewed by Gutiérrez-Alamo et al., 2008). Furthermore, the molecular context of each feed ingredient and nutrient balance can impact nutrient availability. Nutrient requirements of individual animals also vary due to genetics, age, stage of production and environmental factors such as temperature, lighting programs, health status, and even feeding level. Together, these sources of variation pose a great challenge for providing the right nutrients to the right birds at the right time. We must have a solid understanding of the available nutrient content feed and the nutrient requirements of animals in order to match them.

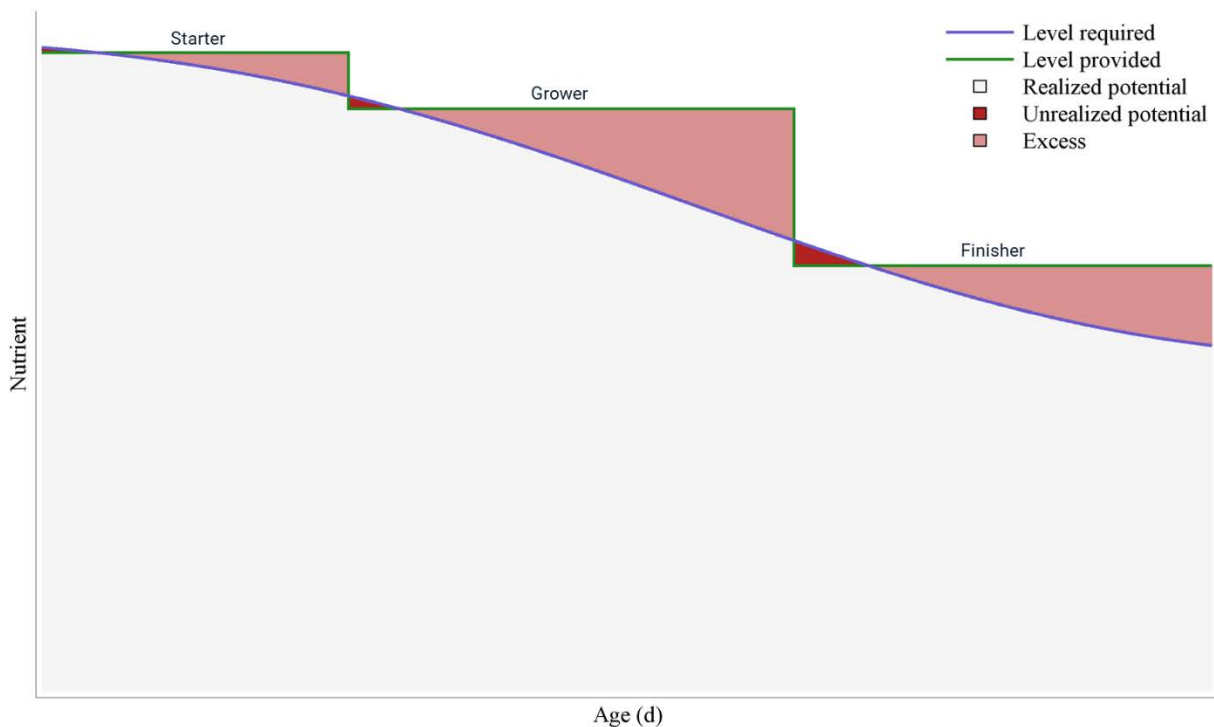


Figure 1. Theoretical flock-level broiler nutrient requirements (purple line), nutrient levels provided by a 3-phase feeding program (green line), and inefficiencies due to excess nutrients (salmon shaded areas) and unrealized growth potential (red shaded areas).

Take Phase Feeding to its Logical Conclusion

Phase names such as pre-starter, starter, grower, developer, finisher, pre-lay, and lay diets are familiar to all nutritionists. Phase feeding is defined as the feeding of several diets, each for a short period of time to roughly meet age-specific nutrient requirements (Figure 1). The point of these diets is to provide approximately the right balance of protein and energy, amino acids, and other nutrients for the birds' age and stage of production. This allows birds to achieve their performance potential while reducing imbalances and excesses that lead to waste. Wasted nutrients are both an economic and an environmental problem.

The number of phases provided is governed by practical considerations. Most barns have limited numbers of feed bins, and the solution has been to deliver a practical quantity to minimize trucking costs, feed the diet until it is depleted, and fill the bin with the next phase diet. Over the years, more phases have been added to reduce nutrient overfeeding. I invite you to suspend the question "How?" for a moment to allow yourself to appreciate the next thought. If you take this phase feeding concept to its logical conclusion, the ideal feeding program to perfectly match nutrient supply in the feed to meet the nutrient requirements of each individual in the flock would be to adjust the feed composition slightly at every meal. Since birds have different temperaments and grow at different rates, measuring these in real-time would help to inform what the diet

composition should be. Repeated measures on individual birds would further inform how they have responded to the nutrients provided previously. Further adjustments could then be made to optimize the formulation to accommodate different nutrient requirements of individual animals.

In practice, some farms have installed feed blending systems that allow them to blend 2 diets. Since the ratio of most nutrients to energy decreases as birds grow, a high nutrient:energy diet can be blended at decreasing ratios with a low nutrient:energy diet over time as the birds grow. Simulation modeling comparing a 3-phase feeding program to a flock-level blending concept demonstrated a reduction in nutrient overfeeding, and a slight reduction in unrealized growth potential. Applying this same concept at an individual bird level suggests that substantial further improvements are possible.

Split Feeding Strategies for Laying Birds

Split feeding can be thought of as a within-day approach to phase feeding. It is a concept that has been used for laying hens. The egg formation process causes highly variable nutritional demands throughout each 24-hour period (Keshavarz, 1998). This process was nicely described and elegantly modeled by Kebreab et al. (2009). Hens typically lay their eggs early in the day, and ovulate shortly thereafter. Whereas yolk is deposited into ovarian follicles on a more or less continuous basis, albumen and shell deposition occur at specific times in a daily pattern. Shell formation, in particular, causes large diurnal fluctuations in Ca requirements. It takes several hours for the egg to be formed to the point where shell is deposited in the shell gland. Until that time, there is no demand for Ca from the bloodstream for eggshell formation. By evening, however, and throughout the night, the Ca demand for eggshell formation increases sharply. Unfortunately, this does not coincide with dietary Ca intake. If Ca is not available from the bloodstream, Ca is mobilized from the skeleton. Since Ca is complexed with P in the bone, this P is also mobilized, but it is in over-supply, and since it cannot be used, it is excreted, with detrimental environmental and cost consequences.

Various strategies have been proposed to enable laying hens to use dietary rather than skeletal Ca. Attempts to increase eggshell quality and minimize bone Ca mobilization include feeding coarse or large particle size Ca (Saunders-Blades et al., 2009; Molnar et al., 2017), midnight feeding (Harms et al., 1996; Lichovnikova, 2007), intermittent lighting programs (Balnave and Muheereza, 1997), and split feeding (Keshavarz, 1998; Molnar et al., 2017; Molnár et al., 2018). Although there are costs associated with storage of multiple feeds and management of a more complex feeding program, the egg quality and profitability benefits are making the concept of split feeding increasingly popular, as a form of within-day phase feeding.

Precision Feeding System for Poultry

Precision feeding (PF) is defined as providing the right amount of the right feed to the right animal at the right time to achieve a desired objective. A well-defined desired outcome dictates the composition and/or quantity of feed required to move an individual from its current state to a preferred state. Sensors inform the system of its current state. A science-based model can be used to estimate what nutrients, if any, should be fed to achieve the desired outcome. Finally, a machine automatically implements the feeding decision to move the animal from its current state closer to the desired outcome.

Recently, my team developed a PF system for controlling feed intake and monitoring BW and feed intake of free-run chickens. Prior systems could monitor feed intake or BW or both. However, our PF system is able to control feed intake. This was a critical technological step toward precision feeding. We have fed broiler breeders the precise quantity of feed required to achieve the desired BW, and have consistently grown group-housed broiler breeders with a BW CV of under 2% at the time of photostimulation (van der Klein et al., 2018a; van der Klein et al., 2018b; Zuidhof, 2018). This was done by measuring each broiler breeder's BW in real-time, and then allowing access to feed only when the birds weighed less than the target BW. We are beginning our first research trial with a new feeding station that allows us to provide a variety of different diets (Figure 2). We will begin by characterizing and modeling the responses of individual birds to a variety of diets with the goal of improving nutrient response models. Ultimately, this new PF system and others like it will be used to provide specific diets based on the attributes of each bird that are measures upon entry.



Figure 2. This second generation precision feeding station controls access to different feeds.

Conclusion

The nutritionist's goal of matching nutrient supply with nutrient requirements at every meal is complex. A lot of data, knowledge, and technology is required. With sensors, however we can detect the current state of the animals within their environment and available nutrient content of feeds. With good models, we can determine the balance of nutrients required by the animal. With

real-time NIR, we can have a pretty good idea of the available nutrient content of feeds and feedstuffs. With the right machines, we can deliver those nutrients to the right bird in real-time. The big data collected in such systems can be used directly to facilitate further improvement and refinement because responses that could formerly only be measured in labour intensive research studies will be collected automatically. By reducing the duration of phase diets to one meal at a time, we will reduce feeding of excess nutrients, and thus reduce excretion of N, P, and CO₂ to the environment, and eliminate the costs associated with providing nutrients in excess. There is good reason to believe that the cost of technology will come down as it has for electronics in general. Precision feeding of poultry may be closer to becoming reality than you think.

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Characterization of Gastric-Intestinal Stability for Processive Endoglucanases as Exogenous Fibre Enzymes in Pigs

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Abstract

Background

Poor utilization of dietary fibre limits pig production profit margin largely due to that current exogenous feed fibre enzymes are originated from *Trichoderma* and *Aspergillus* species with limited stability and efficacy. Two processive endoglucanases, referred to as tCel5A1 and pCel4818_2A, are reported to hydrolyze natural cellulose and have multi-functionality towards hemicelluloses (Basit and Akhtar, *Biotechnology and Bioengineering* 2018, 115:1675; Wang et al., *Scientific Reports* 2019, 9:13630). This study was to investigate the gastric-intestinal stability *in vitro* for these two novel endoglucanases.

Methodology

Cel5A and pCel4818_2A were overexpressed in ClearColi®BL21(DE3). Endoglucanase activity was determined with 0.70% sodium carboxymethyl cellulose and 5 mM dithiothreitol, purged with pressured N₂ and incubated at pH 6.5 – 7.0 and 37 °C for 15 min.

Results and discussion

After incubating the enzymes *in vitro* under the porcine gastric pH (3.5), pepsin activity (274 U/mL) as well as intestinal trypsin (78 U/mL) and chymotrypsin (20 U/mL) activities at pH 6.5 during 0 – 5 h, the Eadie-Hofstee analyses shown that tCel5A1 and p4818Cel5_2A cellulases respectively lost about 18 and 68% of their initial activities after 2 h under the gastric conditions; and lost more than 90% of their initial activities after 2 – 3 h under the intestinal conditions.

Conclusions

Enzyme protein engineering or post-fermentation treatment such as coating for by-passing the gastric-intestinal environment would be needed to enable these two cellulases as efficacious exogenous fibre enzymes.

Applicability and benefits of research to industry

Gastric-intestinal stability should be an important consideration for developing new cellulases and for assessing efficacy of current exogenous feed enzymes in swine dietary applications.

Take home message

Gastric-small intestinal stable and efficacious multifunctional cellulases will dramatically improve dietary fibre utilization and pork production profit.

Key words: fibre, cellulase, pig nutrition

The Effect of Creep Feeding and Nursery Diet Complexity on Growth Performance and Nutrient Digestibility

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Abstract

Suckling piglet growth is dependent on sow milk production. Increased litter size limits milk intake of individual piglets, but creep feeds or milk replacers can be provided as additional nutrient sources and may have benefits post-weaning. This study's objective was to determine if providing creep feed improved growth and the utilization of low complexity diets post-weaning. Fifty-six gilt litters of 12 piglets were recruited. At five days of age, litters were assigned to one of four creep feeding regimens: commercial creep feed (COM), liquid milk replacer (LMR), pelleted milk replacer (PMR), or no creep feed (NO); creep feeds contained 1% brilliant blue as a fecal marker. Individual weights and fecal swabs were collected every 3 days to identify piglets as creep feed consumers. At weaning (21 days of age), six pigs that consumed creep feed were placed on either a HIGH (contained highly digestible animal proteins) or LOW (contained corn and soybean meal as main protein sources) complexity nursery diet in a three-phase feeding program for 38 days.

The LMR disappeared at the greatest rate (37.7 vs. 10.8 ± 1.5 g/pig/d; $P < 0.05$; DM-basis), had the greatest proportion of creep feed consumers (85 vs. $59 \pm 0.4\%$; $P < 0.05$), and the greatest body weight at weaning (6.3 vs. 5.9 ± 0.14 kg). During the nursery period, growth performance was unaffected by creep feeding regimen but pigs fed the LOW diet had reduced average daily gain (25.1 vs. 27.7 ± 0.4 g/kg BW/d; $P < 0.001$), gain:feed (0.75 vs. 0.81 ± 0.02 ; $P < 0.01$), and exit body weight (21.2 vs. 24.4 ± 0.6 kg; $P < 0.001$) versus pigs fed the HIGH diet. At the end of the study, pigs fed LOW nursery diets had lower villus height:crypt depth (1.5 vs. 1.8 ± 0.51 ; $P < 0.05$), but apparent total tract digestibility of organic matter and energy were unaffected by creep feeding or nursery diet regimen. In conclusion, LMR provided during the suckling period resulted in heavier weaning weight but did not improve post-weaning growth or utilization of low complexity nursery diets.

Key words: creep feed, milk replacer, diet complexity, pigs

Punicic Acid as a Potential Antibiotic Replacements in Broiler Diets

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Abstract

The move to reduce antibiotic growth promoters (AGP) in animal diets has hindered the control of enteric disease and reduced broiler performance. Thus, dietary punicic acid (PA) from pomegranate seed oil (PSO) was screened as a potential AGP replacer on performance, necrotic enteritis (NE) intestinal lesions, and footpad dermatitis (FPD) on broilers from 1 to 42 d of age. A total of 1,280 birds were randomly distributed across 8 treatments with 8 replicates of 20 birds each. The treatments were: Positive Control (PC; basal diet with antibiotic), Negative Control (NC; basal diet without antibiotic), and six increasing doses of PA (NC diet + either 0.1, 0.25, 0.5, 1, 1.5, or 2% of the feed). A sub-clinical NE challenge model was applied to all birds (15 X coccidiosis vaccine dose at 12 d and a 24-hour feed removal at 18 d). Feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR) were measured in the starter, grower, and finisher phases. Intestinal NE lesion scores were measured at 20 and 40 d, while FPD scores were measured at 42 d. No significant difference between the PC and NC treatment for performance was found at any phase. Increasing doses of PA quadratically reduced BWG ($P<0.01$), FI ($P<0.01$), and increased FCR ($P=0.01$) before and after the challenge. For the entire period (0 – 41 d), no significant difference for BWG or FCR among treatments was found; however, NC+2% had the lowest FI (85.54 ± 5.54 g/day/bird). The PC treatment had the lowest NE gross lesions, while the treatment NC+2% had the highest number of lesions at 20 d. The NC group had a higher frequency of severe FPD ($P<0.01$) compared with other treatments. The lack of difference between the PC and NC group indicate that the challenge used may not have been severe enough to detect differences in performance and test PA efficacy. The reduction in performance in treatments with higher PA concentrations may be related to other characteristics of PSO. A baker's yeast enriched with PA is being developed to be used in future experiments, eliminating the potential confounding effects of PSO composition. Therefore, PA may still have the potential to be developed as an alternative for the reduced use of AGP in the poultry industry; however, further studies are required to evaluate the exclusive effects of PA in broilers.

Key words: Antibiotic replacers; broilers; necrotic enteritis; performance; punicic acid.

The Effect of Extrusion on Toxicity of Ergot Contaminated Wheat Screenings

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Abstract

Ergot alkaloids (EA) produced by the fungus *Claviceps purpurea*, affect wheat, rye and grasses and have negative effects on livestock if consumed. They exist as R/S-epimers. Research indicates that the R-form is more toxic and feed processing may change epimer ratios. The objective of this trial was to determine if extruding EA contaminated wheat screenings would alter the epimer ratio and reduce toxicity. The experiment utilized a 2 x 2 factorial arrangement of treatments; 0 or 4 ppm EA; processed or not. Wheat screenings (1.1 or 240 ppm EA), were extruded (90°C, 3.2 kPa) and incorporated into diets to achieve 0 (<0.2) or 4 ppm EA. Diets were fed in 2 phases (phase 1, 3wks; phase 2, 5wks) to 160 pigs (65±1 kg initial BW), 5 pigs per pen. Blood samples collected on d7 and 56 were analyzed for prolactin, a sensitive indicator of EA toxicity. Although extrusion had minor effects on total EA content, with the exception of ergometrine, R/S ratios were reduced by extrusion. Extrusion had no effect on average daily gain (ADG), feed intake (ADFI), feed efficiency (FE) or serum prolactin ($P>0.10$) and there were no processing by EA interactions ($P>0.10$). Growth of pigs consuming EA contaminated diets was reduced in phase 1 (1.07, 0.95 kg/d; 0 and 4 ppm, $P<0.001$) and overall (1.07, 1.03 kg/d; 0 and 4 ppm, $P=0.02$). ADFI was reduced in phase 2 (3.22, 3.02 kg/d; 0 and 4 ppm, $P=0.01$). FE was improved when pigs consumed EA contaminated diets in wks 5-6 (0.36, 0.40) and 7-8 (0.30, 0.34; 0 and 4 ppm, $P<0.02$). Serum prolactin (ng/ml) was reduced on d7 (1.67, 0.57; $P<0.001$) and on d56 (0.71, 0.43; $P=0.03$, 0 and 4 ppm) when pigs consumed EA contaminated diets. Consumption of diets contaminated with 4 ppm EA had modest effects on performance of growing pigs, and while extrusion altered the EA epimer profile, it did not mitigate the negative effects. The prolactin results indicate care must be taken to ensure EA contaminated feed is not consumed by lactating sows. Extrusion is not recommended as a solution for EA contaminated grains.

Key words: swine, ergot, extrusion

Evaluation of Intestinal Morphology and Serologic Profiles of Commercially Reared Broilers on Conventional and Alternative Feeding Programs

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Abstract

Antimicrobials are categorized into 4 groups with Category I, II, and III classified as important to human medicine while Category IV has low importance. Chemical coccidiostats are not classified as antibiotics. Canadian broilers may be reared on the following programs: conventional (**CON**, Category III and IV antibiotics and avilamycin permitted), raised without antibiotics of human importance (**RWAOHI**, Category IV antibiotics and avilamycin permitted), or raised without antibiotics (**RWA**, no antibiotics permitted from any category). The objective of this study was to evaluate the impact of program on intestinal morphology and serology of commercial broilers to determine if observed breast yield differences were related to gastrointestinal physiology or systemic metabolism. At 28 days of age, 144 birds/program were sampled from 9 farms in Ontario over 6 consecutive flocks. Birds were bled for plasma, euthanized, weighed, and necropsied for liver weight, small intestine weight, length, and jejunal morphology. Analysis of variance was conducted on effect of program over time (Proc Glimmix, SAS 9.4). Program did not ($P>0.05$) affect BW, small intestine weight or length, jejunal morphology (villus height, crypt depth, VH:CD), plasma proteins or electrolytes. RWA had the largest liver weight compared to CON ($P=0.04$). Plasma aspartate transaminase (**AST**, $P=0.01$) and creatine kinase (**CK**, $P=0.004$) were lowest in RWA compared to CON. Lactate dehydrogenase (**LDH**) was lowest in RWA compared to RWAOHI ($P=0.04$). RWA had the highest alkaline phosphatase (**ALP**, $P=0.05$) and gamma-glutamyl transferase (**GGT**, $P=0.03$). Elevated enzymes in CON birds may be due to protein turnover related to growth rate. Elevated liver weight and GGT in RWA may indicate compromised liver function. Further investigations are required to determine a link between program, morphology and serology on performance.

Key words: antibiotic, intestine, plasma

Protein Cross-Linkage is as an Important Element of the Chemical Changes Associated with Heat Damage to Dietary Proteins

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Abstract

Thermal processing is central to the production of many feed ingredients. Proteins, and the amino acids that compose them, tend to be reactive and can undergo chemical changes during thermal processing. This “heat damage” can have deleterious effects on the nutritional quality of ingredients. The chemical changes associated with heat damage and their impacts on digestibility and bioavailability of amino acids are poorly characterized. Processing of proteins at high temperatures can result in protein cross-linking, the formation of covalent bonds between polypeptide chains within a protein or between proteins. There is emerging evidence that the presence of crosslinks in the structure of proteins, either due to disulphide bridges or cross-linked amino acids, may make parts of these proteins, more resistant to the action of proteases. This study aimed to examine protein cross-linking associated with thermal processing.

Freeze-dried chicken breast meat (CM) samples were subjected to thermal processing at different temperatures (70, 80, 95, 110, 120, and 130°C) for 45 min in an autoclave in absence (Control) or presence of chemical agents. L-serine and ferrous sulfate were used to stimulate amino acid cross-linking (Cross-Linking) and protein oxidation (ProtOx), respectively. Amino acids and cross-linked amino acids, namely lysinoalanine (LAL), lanthionine (LAN) and β -aminoalanine (BAL), were quantified using a standard amino acid analysis technique by UPLC. The content of sulfhydryl (S-H) and disulphide (S-S) bonds in CM was monitored using FT-Raman spectroscopy. Exposing CM samples to increasing temperatures in the absence or presence of L-serine and ferrous sulfate resulted in significant decreases in several essential amino acids (lysine, arginine, histidine, threonine, and methionine) and significant increases in the formation of cross-linked amino acids, notably LAL and LAN. Higher concentrations of cross-linked amino acids were observed in Cross-Linking and ProtOx treatments compared to Control groups. Processing temperatures exceeding 110°C were associated with increased concentrations of LAL and LAN. S-H and S-S bonds contents of CM decreased quadratically in the Control and ProtOx treatment groups with increasing temperature ($p < 0.05$), indicating that the formation of disulphide bridge did not appear to be significant under the conditions examined. This may suggest that cystine in heat damaged proteins can suffer β -elimination of one of the sulfur atoms resulting in the formation of dehydroalanine residues that can react with certain amino acids to form cross-linked amino acids. This study highlights importance of protein cross-linking in heat damage and suggests that cross-linked amino acids can be practical indicators of heat damage for protein ingredients.

Key words: heat damage, proteins, digestibility

The Effect of Replacement of Canola Meal with Enzymatically Modified Canola Meal (ECM) on Growth Performance and Nutrient Utilization of Broiler Chickens

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Abstract

Canola meal (CM) is a valuable source of protein for poultry, despite a presence of substantial amount of non-starch polysaccharides (NSP). Supplementation of poultry diets containing CM with enzymes is an effective mean to improve its nutritive value. The enzymatic hydrolysis of NSP not only improves their digestibility and release some metabolizable energy, but also contributes to the production of prebiotic type NSP hydrolysis products, which might benefit animal health and performance. In our earlier research, the optimal composition and concentration of ingredient-specific carbohydrase enzymes to target the NSP of CM was determined *in vitro*. The most effective enzyme blend containing pectinase, xylanase, and invertase activities lead to depolymerization of 47.9% of NSP of CM and has been selected for the production of the enzyme-modified prebiotic type CM (ECM) for animal studies.

The objective of this study was to evaluate the effects of replacement of CM with a value-added ECM on growth performance, nutrient utilization and gut health of broiler chickens. Four-day-old Ross 308 broiler chickens were assigned to 3 dietary treatments, each consisting of 8 pens of 8 birds each. Birds were raised in electrically heated Super Brooders under controlled environment for 21 days. Birds were fed the Starter and Grower Control diets containing 10% and 20% of CM, respectively, and the experimental diets, where ECM replaced 5% or entire 10% of CM for starter and 20% of CM for grower diets. Body weight and feed intake were recorded on day 4, 14 and 21 of the study. Samples of ileal digesta and excreta were collected to determine the digestibility of nutrients, energy and NSP. Sialic acids, as a components of mucins, was measured to determine the mucin production and gut response to antinutritive properties of fibre components.

Results showed that replacing CM with ECM significantly increased apparent total tract digestibility of NSP and AMEn contents, nitrogen retention in the starter phase, and decreased sialic acid/mucin production in the grower phase. Our research suggests that replacing CM with ECM can improve nutrient digestibility, decrease endogenous losses in poultry due to prebiotic properties of enzymatically released bioactive components of fibre. Enzymatically enhanced CM can improve gut health of broiler chickens and would benefit poultry industry by supporting the antibiotic-free feeding programs.

Key words: carbohydrase, canola meal, enzymatically modified canola meal (ECM), non-starch polysaccharides (NSP), broiler chicken

Impact of A Dietary Citrus Extract on The Behavior and Production of Lactating Dairy Cows Following Regrouping

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Abstract

The objective of this study was to determine if feeding a sensory functional feed additive (mainly based on *Citrus sinensis* extracts) reduces the negative impact of regrouping of lactating dairy cows. Thirty-two, mid-lactation Holstein dairy cows were enrolled as focal cows in this study and housed individually in a tie-stall facility where they were assigned to 1 of 2 treatment diets: 1) control total mixed ration, or 2) control total mixed ration with 4 g/d of citrus extract (Phodé, Terressac, France). Cows were fed their experimental diets for 7 d in the tie-stall facility, then moved to 1 of 2 experimental free-stall pens for a period of 7 d, where they remained on the same treatment diet as provided in the tie-stall facility. Compared with their baseline, primiparous control cows had decreased rumination time on d 1 and 2 and decreased lying time on d 1. In contrast, primiparous cows fed the CE diet did not experience any decrease in rumination and lying time. Primiparous cows fed CE had greater feeding time in the first 2 d after regrouping, while primiparous control cows had greater idle standing time after regrouping. Primiparous CE cows tended to be the recipient of more aggressive interactions at the feed bunk after regrouping than primiparous control cows. However, primiparous CE cows initiated less total competitive behavior after regrouping. Further, primiparous CE cows had greater milk yield as compared with primiparous control cows across the 7 d after regrouping. CE supplementation had little effect on the behavior or production response after regrouping of multiparous cows, who were more experienced with this social stressor. The results indicate that feeding this CE additive to naïve primiparous dairy cows may reduce the negative effects of regrouping on behavior, while promoting greater milk production, after regrouping.

Key words: citrus extract, social regrouping, behavior

Essential Oils to Limit Coccidiosis Impact in Broilers

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Abstract

Antimicrobial alternatives are needed for organic production, but also for limiting drug resistance and complying with upcoming regulations and consumer demands. The aim of this study was to evaluate a mixture of essential oils (EOs) from star anise, thyme, garlic and cinnamon in broilers. Two trials were conducted in non-vaccinated and vaccinated broilers, using an *Eimeria* challenge of 200,000 sporulated oocysts of mixed *Eimeria* species harvested from field isolates. For the first trial, a total of 1,600 non-vaccinated Ross 308 males were allocated to four treatments: 1) noninfected, untreated (NU); 2) infected, untreated (IU); 3) infected, decoquinate-zoalene shuttle program (DZ); 4) infected EOs (EOs). For the second trial, 1,200 coccidiosis-vaccinated Ross 308 males were allocated to the three treatments: 1) NU; 2) IU; 3) EOs. Diets were offered to 8 replicates of 50 chickens for 34 days *ad libitum*. For trial 1, EOs did not affect average daily gain (ADG) or feed conversion ratio (FCR) in the starting (d0-d10) and growing periods (d10-d20), compared to IU birds. However, EOs increased ADG (+9.44 g/d, +9.0%, $P<0.05$) and improved FCR (−0.11, −7.2%, $P<0.05$) in the finishing period (d20-d34), compared to IU birds. At d34, EOs increased body weight (+129 g, +5.7%, $P<0.05$), compared to IU birds. EOs showed the same performance as the conventional anticoccidial program (DZ). For trial 2, EOs increased ADG in the starting (+0.73 g/d, +3.6%, $P<0.05$) and growing periods (+6.69 g/d, +13.6%, $P<0.05$), but not in the finishing period, compared to IU birds. EOs also improved FCR in the starting (−0.07, −5.8%, $P<0.05$) and growing periods (−0.19, −12.3%, $P<0.05$), but not in the finishing period, compared to IU birds. Overall (d0-d34), EOs improved FCR (−0.03, −2.4%, $P<0.05$), compared to IU birds. For the two trials, EOs did not affect oocyst excretion and lesion scores 5- and 6-day post-infection, respectively, compared to IU birds. Overall, the EOs showed performance at the same level of the anticoccidials and improved vaccination in *Eimeria*-challenged broilers. This mixture of EOs could be involved in a strategy to limit coccidiosis impact in broiler production.

Key words: feed additives, coccidiosis, organic, broilers

Diet Nutrient Digestibility and Growth Performance of Weaned Pigs Fed Barley Differing in Fermentable Starch and Fiber

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Abstract

Fermentable starch and fiber in hull-less barley grain are attractive energy sources for weaned pigs due to benefits on gut health. Steam-explosion processing may open the fiber matrix of hulls, increasing the nutritional value of hulled barley. In total, 220 weaned pigs (8.5 kg) were fed 1 of 5 diets containing 59–64% cereal grain: 1) low-fermentable wheat (LFW); 2) low-fermentable hulled barley (LFB); 3) LFB steam-exploded (LFB-E; 1.2 MPa–120s); 4) high-fermentable high β -glucan hull-less barley (HFB); or 5) high-fermentable high amylose hull-less barley (HFA). Diets provided 2.4 and 2.3 Mcal net energy (NE)/kg, 5.5 and 5.1 g standardized ileal digestible lysine/Mcal NE for phase 1 (d 1–14) and phase 2 (d 15–28), respectively. In phase 1, the apparent total tract digestibility (ATTD) of gross energy (GE) and crude protein (CP) was greatest for LFW, intermediate for HFB, followed by LFB-E, LFB and HFA. In phase 2, the ATTD of GE was greater ($P < 0.05$) for LFW and HFB than LFB and LFB-E, and the ATTD of CP was greatest for LFW, intermediate for HFB, followed by LFB, HFA and LFB-E. Predicted NE value was greatest for LFW, intermediate for LFB-E, followed by HFB, LFB and HFA in phase 1, and lowest for HFA among diets in phase 2. For d 1–28, average daily feed intake (ADFI) and average daily gain did not differ among diets, but gain:feed (G:F) for LFB-E was lower ($P < 0.05$) than other 4 diets. Pigs fed LFB and LFW had firmer ($P < 0.05$) feces than pigs fed HFB, HFA and LFB-E. In specific weeks, G:F was lower ($P < 0.05$) for LFB-E than LFW for d 1–14 and ADFI was greater ($P < 0.05$) for LFB-E than HFA for d 22–28. In conclusion, feeding barley instead of wheat grain to weaned pigs reduced nutrient digestibility, and fermentable starch and fiber reduced feces consistency. Steam-explosion did not increase the nutritional value of barley. However, feeding hulled or hull-less barley to replace wheat did maintain growth performance in weaned pigs.

Key words: barley, fiber, starch

Antimicrobial Resistance Phenotype and Genotype of Generic *Escherichia coli* from Encapsulated Cinnamaldehyde and Citral Fed-Broiler Chicken

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Abstract

This study investigated the effects of in-feed encapsulated cinnamaldehyde (CIN) and citral (CIT) alone or in combination (CIN+CIT) on antimicrobial resistance (AMR) phenotypes and genotypes of *E. coli* isolated from feces of 6-, 16-, 23-, and 27-days old broiler chickens. The dietary treatments included basal diet (NC), the basal diet supplemented with 55 ppm bacitracin (BAC), 100 encapsulated CIN, CIT, or CIN+CIT. In general, the prevalence of resistance to 10 antimicrobials was lower in the isolates from CIN compared to those from NC or BAC ($P < 0.05$). The whole genome sequencing analysis of 227 of the 240 isolates detected 26 AMR genes (ARGs) and 19 plasmids but the prevalence of some ARGs and plasmids was lower in *E. coli* isolated from CIN or CIN+CIT than NC or BAC ($P < 0.05$). The most prevalent ARGs included *tetA* (n=108), *aac3_Vla* (n=91), *aadA1* (n=86), *bla_{CMY-2}* (n=78), *sul1* (n=77), *aph3_lb* (n=58), *aph6_ld* (n=58), and *sul2* (n=24). Interestingly, the number of most virulence genes (VGs) increased over time from 6 to 27 days of age ($P < 0.05$). The prevalence of isolates of serotype O21:H16 was lower in CIN and CIN+CIT while colibacillosis-associated multi-locus sequence types (ST117) were the most prevalent in the isolates from day 23 ($P < 0.05$). A whole genome based phylogenetic tree revealed a close relationship of 25 of 227 isolates to human or broiler extraintestinal pathogenic *E. coli*. In conclusion, this study indicates that AMR and virulence genotype of *E. coli* could be affected by encapsulated CIN or CIN+CIT feed supplementations.

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Performance and Environmental Benefits from Biochar Supplementation in Beef Cattle Grazing Systems

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Abstract

Reducing greenhouse gas emissions from beef cattle production systems continues to be a pressing matter within the beef industry. The objectives of a series of experiments conducted were to: (1) determine the optimum dose of biochar for reducing methane emissions in beef cows. (EXP1) and (2) determine whether biochar supplementation can reduce methane emissions from cows on pasture (EXP2). In EXP1, pregnant beef cows (n=8) of similar weight and stage of pregnancy were enrolled in a Latin square study with biochar (Oregon Biochar Solution, White City, OR) supplemented at 0, 1, 2, or 3% of total dry matter intake (DMI). Each period consisted of 21 d: 14 d adaptation and 7 d for data collection. Enteric gas emissions were measured using C-Lock GreenFeed trailers (C-Lock Inc., Rapid City, SD, USA) and DMI was measured using Insentec feeders (Insentec, Voorsterweg, The Netherlands). In EXP2, 64 cows were divided into 8 paddocks of 8 cow-calf pairs. Using a crossover design, each paddock was assigned to either control (no biochar) or biochar supplemented at 3% of estimated DMI. Enteric gas emissions from each paddock were measured as in EXP1 during the last week of each 4-week period. Pasture DMI was estimated using paddock entry/exit quadrats during each sampling week. Enteric methane emissions expressed in g CH₄/day were not affected by biochar supplementation in EXP1 ($P \geq 0.56$; although the 3% inclusion was numerically lowest) or EXP2 ($P \geq 0.37$). Feed intakes ($P \geq 0.15$) and cow weights ($P \geq 0.34$) were also not affected by biochar supplementation in either experiment. Results suggest that biochar was not effective for reducing methane emissions from beef cows fed a forage diet or on pasture; however, animal performance was not affected. Further work is required to determine if type or higher inclusions of biochar can reduce methane emissions from grazing beef cattle.

Key words: biochar, methane, beef cattle, pasture

Soil Organic Carbon Content in a Naturalized Perennial Pasture Compared to an Annual Silage Cropping System Over a 15-year Time Period in Maritime Canada

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Abstract

Soil organic carbon (SOC) is a large and dynamic pool which offsets greenhouse gas (GHG) emissions from agriculture that is affected by pasture management and is influenced by agricultural practices. Decades of tillage and poor soil health practices have reduced historical soil carbon stocks. Implementing land use practices which promote an influx and accumulation of soil organic carbon have become increasingly important to improve soil health and offset GHG emissions. Permanent perennial pasture has been shown previously to improve SOC stocks, while annual cropping practices can reduce soil organic carbon stocks. Historical soil data (0-15cm depth) of two adjacent fields both managed as naturalized perennial pasture were compared to three adjacent fields all under annual corn and forage silage management at the AAFC Research Farm in Nappan, NS, Canada. A mixed effects model of the historical soil carbon data was done for four separate years (2001, 2002, 2012 & 2016) in which the management practices did not change. The naturalized perennial pasture treatment had significantly higher ($P<0.05$) soil organic carbon stocks (27.3 ± 0.89 g/kg DM) than the annual silage treatment (21.8 ± 0.77 g/kg DM); however, there was no effect of year or year x treatment interaction on SOC for either treatment. This research demonstrates that naturalized perennial pasture in Maritime Canada can maintain a significantly higher concentration of SOC in comparison to an annual silage cropping system. This could have implications for farmers looking for strategies to increase their soil organic carbon stocks and simultaneously improve soil health.

Predicting Live Weight Using Linear Measurements in Growing Beef Calves

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Abstract

Body weights provide an insight of estimation of growth, health, nutrient requirements, and efficacy of cattle. However, few producers have scales or labour resources to routinely weigh cattle. New visual technology systems coupled with machine learning algorithms may be able to address these challenges, however links between linear measurements and body weight (BW) need to be established. To meet these objectives, a total of 103 calves (n=103), 57 females and 46 male Angus cross calves were enrolled in the study. Calves were weighed and measured weekly from two weeks to eight weeks of age. Linear measurements included: poll to nose, width across the eyes (WE), width across the right ear, neck length, wither height, heart girth (HG), midpiece height, midpiece circumference, midpiece width, midpiece depth, hook height, hook width, pin height, top of pin bones width (PW), width across the ends of pin bones, nose to tail body length, the length between the withers and pins, forearm to hoof, cannon bone to hoof. These measurements were taken using commercial soft tape measure and calipers. To assess relationships between traits and to fit a model to predict BW, data was analyzed using Weka (The University of Waikato, New Zealand) software using both Linear Regression (LR) and Random Forest (RF) models. The LR model used 11 traits to fit the data ($r^2 = 0.97$) where the highest coefficients were HG, PW and WE. The RF model improved further the BW predictions ($r^2 = 0.98$). While HG is a well-known biometric measurement known to correlate well with BW, it cannot be directly estimated using 3D Computer Vision (CV) technologies. The WE could be a potentially great candidate for new BW prediction models based on CV-acquired information. The results of this study suggest using linear measurements can accurately estimate body weight in beef calves. These models may be able to help producers and nutritionists accurately estimate BW when a scale is not available and may assist with the development of advanced CV-based weighing system for calves.

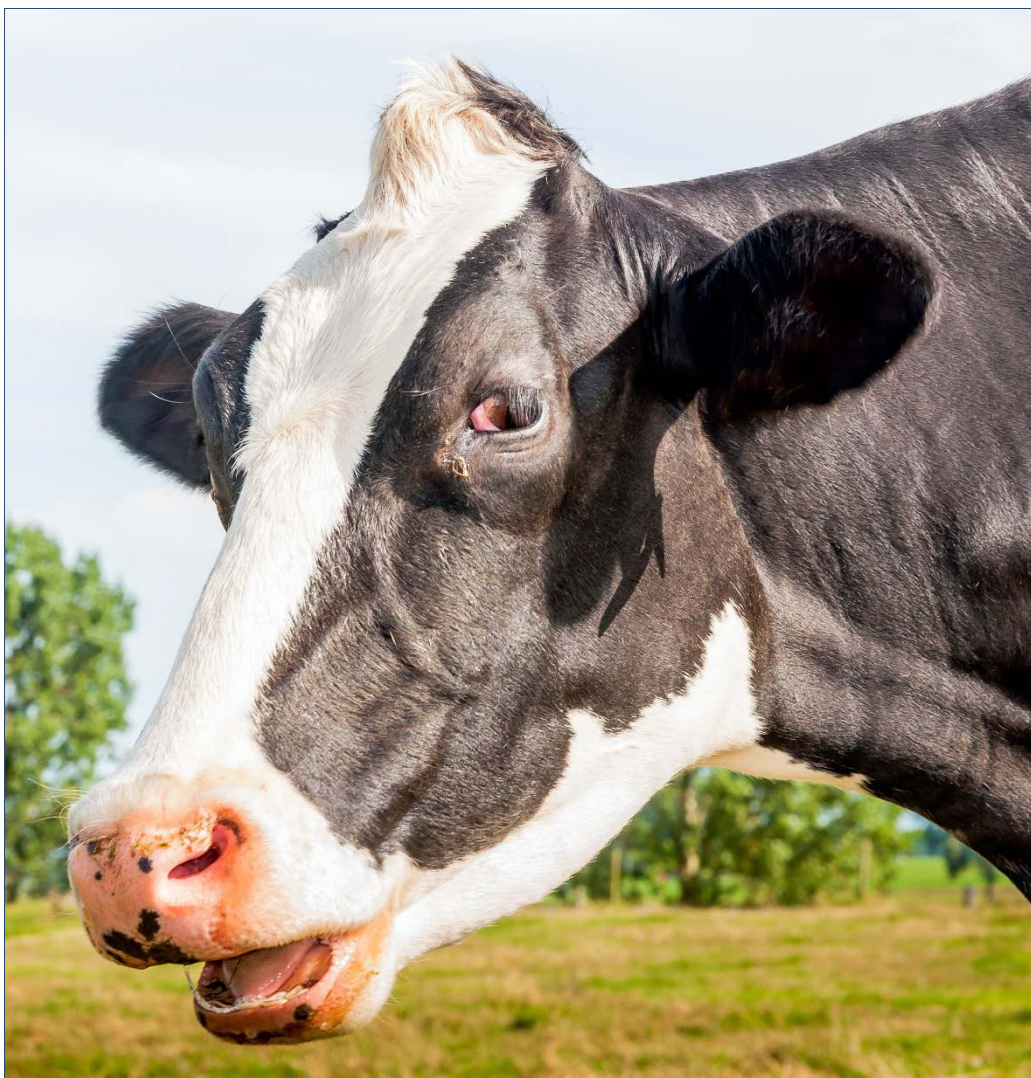
Effects of Grass Hay vs. Silage-Based TMR on Performance of Dairy Calves around weaning

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Abstract

Historically milk fed dairy calves have not been given access to forage during the milk feeding period. However, the move towards feeding higher milk volumes has resulted in renewed interest in providing milk fed calves access to forage. The aim of this study was to compare feed intake and weight gain of dairy calves (n=108) offered two different forages and weaned using three gradual weaning methods. Weaning treatments were assigned to calves within each pen, and each pen was alternately assigned to Hay (mix of tall fescue, orchard grass) or a total mixed ration (lactating cow TMR: 46% concentrate, 32% corn silage, 14% grass silage, 6% alfalfa hay, 2% straw). All calves were offered 12 L/d of milk until d 30; milk was then gradually reduced by 25% of the individual's average milk intake over 3 d. Milk was further reduced when calves either reached d 62 of age (milk reduced linearly until weaned at d 70), when each calf reached 3 specific forage and starter DMI targets (25% of milk removed at 200 g DM/d, 600 g DM/d, and weaned at 1150 g DM/d), or when each calf reached 200 g DM/d (milk reduced linearly until weaned at d 70). During weaning (d 31-69), Hay and TMR calves ate similar amounts of forage (0.09 vs 0.08 ± 0.01 kg DM/d, respectively) but Hay calves ate more starter (0.85 vs 0.65 ± 0.09 kg DM/d, respectively). Postweaning (d 70-84), Hay calves continued to consume more starter (2.81 vs 2.39 ± 0.1 kg DM/d, respectively) while TMR calves consumed more forage (0.3 vs 0.1 ± 0.02 kg DM/d, respectively). Over the 84 d calf rearing period, Hay calves consumed more DM than TMR calves (126.1 vs. 112.6 ± 3.5 kg DM, respectively), which likely explains the higher final BW of Hay calves (118.5 vs 112.7 ± 1.7 kg, respectively). There was no interaction between forage treatment and weaning method for feed intakes or final BW. We conclude that feeding grass hay over a silage-based TMR can promote starter intake and BW in calves fed a high milk allowance and weaned gradually. We also provide evidence that, despite historical beliefs, high milk fed calves can be offered forage during the milk-feeding period and still maintain high levels of performance.

Key words: forage, gradual weaning, weight gain



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