



**2019 ANIMAL NUTRITION  
CONFERENCE OF CANADA**



**2019 COLLOQUE DE NUTRITION  
ANIMALE DU CANADA**

## Proceedings

Cahier de conférences



# 14-16

May/mai 2019

**Niagara Falls**  
Ontario

**Integrating epigenetic concepts  
and principles with animal nutrition**

Intégration des concepts et principes  
épigénétiques à la nutrition animale

**Sponsor, pre-conference symposium**  
Commanditaire, symposium pré-colloque





## Welcome from ANAC / Bienvenue de l'ANAC

The Animal Nutrition Association of Canada (ANAC) is honoured to organize and host the Animal Nutrition Conference of Canada (ANCC). The feed industry continues to face changing consumer trends and customer requirements, new regulations, as well as new challenges related to antimicrobial use and how we raise livestock and poultry. Having a forum where the industry can learn about the newest scientific developments and connect with colleagues and stakeholders from across the country and beyond to learn and exchange ideas is more important than ever. This is reflected in the move of the conference from Guelph to Niagara Falls, a location that could accommodate our more than 300 participants. ANAC is pleased to once again host a regulatory session directly following the conference, to provide an introduction to the anticipated overhaul of the *Feeds Regulations* that govern our Canadian industry.

The conference organizing committee has planned an exceptional program and ANAC would like to thank them for their dedication over the past year. We would also like to thank our industry partners, as a conference of this caliber is not possible without your generosity and support. ANAC would also like to express our gratitude to the world-class speakers who will be sharing their knowledge and research over the next couple of days. Finally, we have a record number of graduate student posters on display this year which is a testament of the significant research in animal nutrition being conducted in Canada.

We hope you enjoy the third edition of the ANCC and take advantage of the many opportunities to grow your knowledge as well as your network of friends and colleagues.

*Melissa Dumont, agr.*

Executive Director, Animal Nutrition Association of Canada

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*L'Association de nutrition animale du Canada (ANAC) a l'honneur d'organiser et d'être l'hôte du Colloque de nutrition animale du Canada (CNAC). L'industrie de l'alimentation animale continue d'être confrontée à l'évolution des tendances de consommation et des exigences des consommateurs, aux nouveaux règlements, ainsi qu'aux nouveaux défis liés à l'utilisation des antimicrobiens et à la façon dont nous élevons le bétail et la volaille. Il est plus important que jamais d'avoir un forum où l'industrie peut se renseigner sur les plus récents développements scientifiques et communiquer avec des collègues et des intervenants de partout au pays et d'ailleurs pour apprendre et échanger des idées. Cela se reflète dans le déménagement du colloque de Guelph à Niagara Falls, un endroit capable d'accueillir plus de 300 participants. L'ANAC est heureuse de tenir une fois de plus une séance de réglementation immédiatement après le colloque, afin de livrer les résultats de la refonte du Règlement sur les aliments du bétail qui régit notre industrie canadienne.*

*Le Comité organisateur du colloque a préparé un programme exceptionnel et l'ANAC remercie ses membres pour leur dévouement à la cause au cours de la dernière année. Nous tenons également à remercier nos partenaires de l'industrie puisqu'un colloque de ce niveau ne peut avoir lieu sans leur générosité et leur appui. L'ANAC aimerait également exprimer sa gratitude aux conférenciers de classe mondiale qui partageront leurs connaissances et leurs recherches au cours des deux prochains jours. Enfin, nous avons un nombre record d'affiches d'étudiants diplômés exposées cette année, ce qui témoigne de l'importance de la recherche en nutrition animale qui se poursuit au Canada.*

*Nous espérons que vous aimerez la troisième édition du CNAC et que vous saurez profiter des nombreuses occasions d'accroître vos connaissances et d'élargir votre réseau d'amis et de collègues.*

*Melissa Dumont, agr.*

Directrice exécutive, Association de nutrition animale du Canada

## Organizing Committee ANCC 2019 / Comité Organisateur 2019

We are honoured to welcome you to the 3rd annual Animal Nutrition Conference of Canada (ANCC). Over the past three years, the ANCC has brought together researchers and feed industry specialists for a dynamic exchange of knowledge about the latest scientific developments in livestock and poultry nutrition. This year, we have selected the theme “Integrating epigenetic concepts and principles with animal nutrition”. Technology and scientific concepts in animal agriculture are advancing. To continue to improve how we manage and feed animals, there must be a connection between scientific concepts and applied use in the field. We know that certain changes at the parental level can impact offspring characteristics -- in addition to outside factors such as diet, environment and microbes. Epigenetics is an innovative science which goes beyond everyday nutritional requirements. The study of epigenetics helps us understand changes happening at the molecular level, with different modifications that do not alter the genetic code itself, and how this can influence what we see in the field.

The committee sought to bring together Canadian and global experts from across the human and agriculture sectors to present an introduction to this cutting-edge topic and the shape of our knowledge to date. The goal of the conference is to highlight innovative and applicable research as well as showcase student researchers and industry partners, providing lots of opportunities for learning and networking. Thank you to our conference sponsors and participants for supporting this event. Have fun at ANCC 2019!

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*Nous sommes heureux de vous accueillir au 3e Colloque annuel de la nutrition animale du Canada (CNAC). Depuis trois ans, des chercheurs et des spécialistes de l'industrie de l'alimentation animale se réunissent lors du colloque pour partager leurs connaissances sur les plus récentes découvertes scientifiques relatives à l'alimentation du bétail et de la volaille. Cette année, le colloque porte sur le thème « Intégration des concepts et principes épigénétiques à la nutrition animale ». La technologie et les concepts scientifiques en agriculture animale progressent. C'est ainsi que pour continuer d'améliorer notre façon de gérer et de nourrir les animaux, nous devons établir un lien entre les concepts scientifiques et leur application sur le terrain. Nous savons qu'en plus des facteurs externes, comme l'alimentation, l'environnement et les microbes, certains changements chez les parents peuvent avoir un effet sur les caractéristiques de leurs descendants. L'épigénétique est une discipline novatrice qui explore beaucoup plus que les exigences alimentaires quotidiennes. L'étude de l'épigénétique nous aide à comprendre les différents changements moléculaires sans toutefois modifier le code génétique lui-même et la façon dont ceux-ci peuvent influencer ce que nous retrouvons sur le terrain.*

*Le comité a tenté de réunir des experts canadiens et étrangers provenant des milieux agricole et humain pour introduire cette discipline de pointe et les connaissances acquises jusqu'à présent. Le colloque vise à mettre en valeur les percées novatrices et applicables. Il offre également une formidable vitrine pour les recherches menées par des étudiants diplômés et les partenaires de l'industrie. Les occasions d'apprentissage et de réseautage seront nombreuses. Nous remercions les commanditaires de cet événement ainsi que les participants pour leur précieux soutien. Nous vous souhaitons un excellent CNAC 2019!*

**Kayla Price**, Alltech Canada  
(Program Chair/Présidente du programme)

**Theunis Wessels**, Lallemand Animal Nutrition  
(Sponsorship Chair/Président des commanditaires)

**Elijah Kiarie**, University of Guelph  
(Academic Chair/Président de l'académie)

**Jeff Bond**, PMT Inc.

**Trevor DeVries**, University of Guelph

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**Kathleen Shore**, New Life Mills, a Division of Parrish  
& Heimbecker

**Tom Wright**, Ontario Ministry of Agriculture & Rural  
Affairs (OMAFRA) / University of Guelph

**My-Lien Bosch**, Animal Nutrition Association of Canada  
(ANAC)

**Josée Lafontaine**, Animal Nutrition Association  
of Canada (Logistics/Administration)

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**Integrating epigenetic concepts and principles with animal nutrition**  
**Intégration des concepts et principes épigénétiques à la nutrition animale**

\*All events will be taking place on the 3<sup>rd</sup> Floor of the Sheraton on the Falls Hotel.

\*Tous les événements auront lieu au 3<sup>e</sup> étage de l'hôtel Sheraton on the Falls.

———— **TUESDAY, MAY 14<sup>TH</sup> – 4:30 PM TO 6:30 PM / MARDI LE 14 MAI - DE 16H30 À 18H30** ————

**WELCOME COCKTAIL / COCKTAIL DE BIENVENUE** SPONSORED BY / COMMANDITÉ PAR KEMIN

Join us for some animated discussions and meet with industry associates with a spectacular view of the Falls.  
 Registration packages will also be ready for pickup.

Joignez-vous à nous pour des discussions animées, et rencontrez des associés de l'industrie tout en profitant d'une vue spectaculaire sur les chutes. Vous pourrez aussi récupérer votre trousse d'inscription.

———— **WEDNESDAY, MAY 15<sup>TH</sup> – MORNING / MERCREDI LE 15 MAI - MATINÉE** ————

Conference registration begins at 7 AM and continues throughout the day.

L'inscription au colloque débute à 7 H et se poursuit toute la journée.

<b>PRE-CONFERENCE SYMPOSIUM</b> <b>SYMPOSIUM PRÉ-COLLOQUE</b>  <b>Nutritional Health - Investing in Innovation for Livestock and Poultry</b> <b>Santé Nutritionnelle - Investir dans l'innovation pour le bétail et la volaille</b> <b>GREAT ROOM B&amp;C / GRANDE SALLE B&amp;C</b>		
7:15	Hot breakfast / Petit-déjeuner chaud	
8:05	Introduction	<b>André Gilbert</b>
	Session Chair / Président de séance	<b>Dr. Haitham Yakout</b>
8:15	Advances in development and application of direct fed microbials for monogastric food animals <i>Progrès réalisés dans le développement et l'utilisation de produits microbiens à administration orale pour les animaux monogastriques destinés à l'alimentation</i>	<b>Dr. Adam Nelson</b> Novozymes
8:55	Butyrate : signaling potential along the GIT and impact on poultry and swine productions <i>Butyrate : potentiel de signaux le long du tractus digestif et impact sur les productions de volaille et de porc</i>	<b>Dr. Tim Goossens</b> Adisseo
9:35	The effects of diet and epigenetic alterations on the gut microbiome, inflammation and poultry production <i>Effets des altérations diétiques et épigénétiques sur le microbiome de l'intestin, sur l'inflammation et sur la production de volaille</i>	<b>Dr. Michael H. Kogut</b> USDA-ARS
10:15	Health break / Pause-santé	
	Session Chair / Présidente de séance	<b>Dr. Maris McCarthy</b>
10:40	Transition cow nutrition : setting her up for success <i>Nutrition de la vache en période de transition : assurer la réussite</i>	<b>Dr. Joe McFadden</b> Cornell University
11:20	Improving immunity in dairy cows during times of stress through nutrition <i>Utiliser la nutrition pour améliorer l'immunité chez les vaches laitières en période de stress</i>	<b>Dr. Michael Ballou</b> Texas Tech University
	Closing remarks / Propos de clôture	<b>Felipe Navarro</b>
12:05	Lunch & visit of student posters / Dîner et visite des affiches produites par les étudiants	

<b>OPENING PLENARY / PLEINIÈRE D'OUVERTURE</b> <b>GREAT ROOM B&amp;C / GRANDE SALLE B&amp;C</b>		
1:05	Opening remarks from ANAC <i>Propos d'ouverture de l'ANAC</i>	<b>Melissa Dumont</b> ANAC
1:10	Organizing Committee welcome <i>Mot de bienvenue du comité organisateur</i>	<b>Dr. Kayla Price</b> Alltech Canada
1:15	The impact of '-omics' technologies on nutrition <i>L'impact des technologies en «-omique» sur la nutrition</i>	<b>Dr. Chris Ashwell</b> North Carolina State University
2:00	<b>Kees de Lange Lectureship in Animal Nutrition, SPONSORED BY THE UNIVERSITY OF GUELPH</b> , Microbial sensing : implications for gut health in the neonate <b>Conférence commémorative en nutrition animale Kees de Lange, COMMANDITÉ PAR L'UNIVERSITÉ DE GUELPH</b> , Détection des microbes : répercussions sur la santé intestinale des nouveau-nés	<b>Dr. Andrew Van Kessel</b> University of Saskatchewan
3:00	Health break / Pause-santé	
3:30	Microbial endocrinology : Why the evolutionary-based integration of microbiology and neurobiology matters in the examination of the intersection of animal nutrition epigenetics <i>Endocrinologie microbienne : pourquoi l'intégration de la microbiologie et de la neurobiologie basée sur l'évolution compte dans l'examen de l'intesection de la nutrition animale et de l'épigénétique</i>	<b>Dr. Mark Lyte</b> Iowa State University
4:15	How can early social environment impact epigenetics, health and inform choices <i>Comment l'environnement social précoce in luence l'épigénétique, la santé et éclaire les choix</i>	<b>Dr. Moshe Szyf</b> McGill University
5:00	<b>ANAC Scholarship announcement and recipient's presentation of research</b> : Effect of wheat straw chop length in a high-straw dry cow diet on intake, behaviour and health of dairy cows <b>Annonce de la bourse d'études de l'ANAC et présentation de la récipiendaire sur sa recherche</b> : Effet de la longueur des particules de paille et blé dans une ration riche en paille pour vaches taries sur la consommation, le comportement et la santé des vaches laitières	<b>Casey Havekes</b> University of Guelph Recipient / Lauréate
5:15	End of opening plenary / Fin de la plénière d'ouverture	
5:15	<b>ANCC 2019 Reception</b> : Come and enjoy an evening of food, drinks and networking around the industry partner showcase and graduate student poster competition.	
7:15	<b>Réception du CNAC 2019</b> : Venez profiter d'une soirée où gastronomie et réseautage seront à l'honneur, à l'occasion du salon des partenaires de l'industrie et du concours d'affiches pour étudiants diplômés.	

**THURSDAY, MAY 16<sup>TH</sup> - MORNING / JEUDI LE 16 MAI - MATINÉE**

Conference registration begins at 7 AM and continues throughout the day.

*L'inscription au colloque débute à 7H et se poursuit toute la journée.*

**Concurrent Sessions / Séances simultanées**

<b>MONOGASTRIC SESSION / SÉANCE SUR LES MONOGASTRIQUES</b>		
<b>GREAT ROOM B / GRANDE SALLE B</b>		
7:15	Hot breakfast / <i>Petit-déjeuner chaud</i>	
8:10	Opening remarks by Session Chair <i>Propos d'ouvertures du Président de séances</i>	<b>Rob Patterson</b> Canadian Bio-Systems Inc.
8:15	Influence of dietary micro-minerals on the intestinal health of broilers <i>Influence des micro-minéraux sur la santé enterique des poulets à griller</i>	<b>Dr. Todd Applegate</b> University of Georgia
9:00	Amino acid nutrition : long-term implications for sows and offspring <i>Nutrition et aminoacides : implication à long terme pour les truies et pour leur progéniture</i>	<b>Dr. Lee-Anne Huber</b> University of Guelph
9:45	Health break / <i>Pause-santé</i>	
10:15	Effects of diet on gastrointestinal microbial ecosystems and gut gene expression in young pigs <i>Effets du régime sur l'écosystème microbien gastro-intestinal et sur l'expression génétique de l'intestin chez les jeunes porcs</i>	<b>Dr. Nuria Canibe</b> Aarhus University, Denmark
11:00	<i>In ovo</i> and neonatal nutrition in poultry <i>Nutrition de l'embryon et du nouveau-né chez la volaille</i>	<b>Dr. Peter Ferket</b> North Carolina State University
11:45	Precision feeding of gestating first parity sows improves sow body weight gain in late gestation <i>L'alimentation de précision des truies primipares gestantes améliore le gain de poids corporel en fin de gestation</i>	<b>Victoria Stewart</b> University of Guelph Graduate student / <i>Étudiante diplômée</i>
12:00	Lunch / <i>Dîner</i>	

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**THURSDAY, MAY 16<sup>TH</sup> - MORNING / JEUDI LE 16 MAI - MATINÉE**

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Conference registration begins at 7 AM and continues throughout the day.

*L'inscription au colloque débute à 7H et se poursuit toute la journée.*

**Concurrent Sessions / Séances simultanées**

<b>RUMINANT SESSION / SÉANCE SUR LES RUMINANTS</b>		
<b>GREAT ROOM C / GRANDE SALLE C</b>		
7:15	Hot breakfast / <i>Petit-déjeuner chaud</i>	
8:10	Opening remarks by Session Chair <i>Propos d'ouvertures du Président de séances</i>	<b>Dr. Trevor DeVries</b> University of Guelph
8:15	Feeding for dual purpose with dual benefit: role of nutrition during pregnancy on cow and calf health and performance <i>L'alimentation à double objectif et double bénéfice : rôle de la nutrition durant la grossesse sur la santé et le rendement de la vache et du veau</i>	<b>Danielle Coleman</b> <b>Dr. Juan Loor</b> University of Illinois
9:00	The effects of nutrient supply during gestation on maternal, fetal and postnatal outcomes in ruminants : Emphasis on early pregnancy <i>Les effets de l'apport en nutriments durant la gestation sur les résultats maternels, foetaux et postnataux chez les ruminants</i>	<b>Dr. Joel Caton</b> North Dakota State University
9:45	Health break / <i>Pause-santé</i>	
10:15	Interaction of nutrition and epigenetic effects in dairy cattle <i>Interaction entre la nutrition et les effets épigénétiques chez les bovins laitiers</i>	<b>Dr. Alex Bach</b> ICREA <sup>1</sup> & IRTA <sup>2</sup> , Spain
11:00	Developmental programming in the beef industry <i>Programmation de la croissance dans l'industrie du boeuf</i>	<b>Dr. Katie Wood</b> University of Guelph
11:45	Embryonic response to high beta-hydroxybutyrate (BHB) levels in postpartum dairy cows <i>Réponse embryonnaire à des niveaux élevés en bêta-hydroxybutyrate (BHB) chez la vache laitière en début de lactation</i>	<b>Catherine Chaput</b> Université Laval Graduate Student / Étudiante diplômée
12:00	Lunch / <i>Dîner</i>	

<sup>1</sup>Catalan Institution for Research and Technology in Agrifood

<sup>2</sup>Institute for Research and Technology in Agrifood

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**THURSDAY, MAY 16<sup>TH</sup> - AFTERNOON / JEUDI LE 16 MAI - APRÈS-MIDI**

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<b>CLOSING PLENARY / PLÉNIÈRE DE CLÔTURE</b>		
<b>GREAT ROOM B&amp;C / GRANDE SALLE B&amp;C</b>		
1:15	'Epi-nutrigenomics': epigenetic mechanisms as links between nutrition and performance in livestock <i>L'«épig-nutrigénomique» : mécanismes épigénétiques permettant de créer des liens entre la nutrition et la performance chez le bétail</i>	<b>Dr. Hélène Jammes</b> INRA (French National Institute for Agricultural Research)
2:00	The impact of early nutritional insults and long-term consequences on health <i>Effets des carences nutritionnelles en bas âge et conséquences durables pour la santé</i>	<b>Dr. Robert Bertolo</b> Memorial University of Newfoundland
2:45	Closing remarks by Session Chair <i>Propos de clôture de la Présidente de séances</i>	<b>Kathleen Shore</b> New-Life Mills

ANAC will be hosting a session on Canadian regulatory updates from 3:15 PM to 4:45 PM, after a short break, following the closing remarks.

*L'ANAC présentera une séance sur la mise à jour de la réglementation canadienne, de 15h15 à 16h45, après une petite pause, peu de temps après la Plénière de clôture.*

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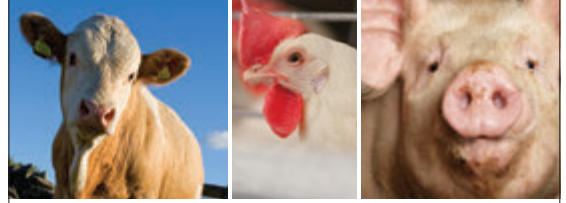
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# Pre-Conference Symposium

## Symposium précolloque





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## **Advances in Development and Application of Direct Fed Microbials for Monogastric Food Animals**

### **Progrès dans le développement et l'utilisation de produits microbiens à administration orale pour les animaux monogastriques destinés à l'alimentation**

*Adam Nelson, PhD*  
*Senior Scientist, Novozymes Biologicals*  
*5400 Corporate Circle, Salem, VA 24153 United States*  
*adnx@novozymes.com*

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#### **Abstract**

Probiotics have been increasingly used to improve animal health and enhance nutritional efficiency. The need for probiotic solutions is driven by several factors, including replacements for antibiotic growth promoters, and improved consumer awareness. Understanding how probiotics work is challenging, but has been aided recently by technology-driven approaches. This has allowed researchers to discover links between the host, the resident microbiome, and the probiotic. The development of *Bacillus subtilis* DSM 29784 as a probiotic for broilers is an example of the application of a direct fed microbial to improve health, and serves as a platform for applying several tools to understand mechanism of action.

#### **Résumé**

On utilise de plus en plus les probiotiques pour améliorer la santé animale et le rendement nutritionnel. La nécessité de solutions probiotiques découle de plusieurs facteurs, incluant le remplacement des antibiotiques promoteurs de croissance et les consommateurs mieux informés. Comprendre comment les probiotiques agissent n'est pas facile, mais des approches technologiques nous aident depuis peu à y voir plus clair. Cela a permis aux chercheurs de découvrir les liens qui existent entre l'hôte, le microbiome résident et le probiotique. Le développement de *Bacillus subtilis* DSM 29784 comme probiotique pour les poulets à griller est un exemple d'administration orale d'un produit microbien pour améliorer la santé, servant de plate-forme pour appliquer plusieurs outils

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## **Butyrate: Signaling Potential Along the GIT and Impact on Poultry and Swine Productions**

### **Le butyrate : potentiel de signaux le long du tractus digestif et impact sur les productions de volaille et de porc**

*Dr. Tim Goossens<sup>1</sup>*

*<sup>1</sup> Global Scientific & Technical Manager, Adisseo, France; tim.goossens@adisseo.com*

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#### **Abstract**

Several reports have been published describing positive effects of endogenous and exogenous butyrate on gut health, supporting the use of butyrate products as feed supplements to production animals. Butyrate is a molecule that is well known for its ability to elicit numerous effects at the cellular or microbiological level in digestive as well as extra-intestinal tissues. It can be used as a cellular energy source, but it also has distinct effects on gene expression: butyrate can bind to specific receptors to induce several signal transduction cascades, in addition to epigenetically affecting gene expression through its function as an histone deacetylase inhibitor (HDACi).

Apart from giving an overview of the wide range of signaling processes butyrate can modulate, I will present data demonstrating that the effects of supplementing livestock animals with dietary butyrate are heavily dependent on the enteric location where ingested butyrate is delivered in the digestive tract of animals. For example, in at least some conditions, elevated butyrate concentrations in the fore- and midgut of broilers may induce negative effects on caecal microbiota diversity and/or inflammation, as opposed to increased butyrate concentrations in the hindgut.

It is therefore of critical importance to investigate the butyrate release kinetics of different commercially available butyrate products, to explain the distinct effects they can have on the health and performance of production animals.

#### **Résumé**

Plusieurs rapports ont été publiés décrivant les effets positifs du butyrate endogène et exogène sur la santé de l'intestin et appuyant l'emploi de produits de butyrate comme suppléments dans les aliments destinés aux animaux d'élevage.

Le butyrate est une molécule bien connue pour sa capacité à élucider plusieurs effets au niveau cellulaire ou microbiologique dans les tissus digestifs et les tissus extra-intestinaux. Il peut servir de source énergétique cellulaire, mais il a aussi des effets distincts sur l'expression des gènes: le butyrate peut s'agglutiner à des récepteurs spécifiques pour induire plusieurs cascades de transduction des signaux, en plus d'affecter à l'échelle épigénétique l'expression des gènes à travers sa fonction d'inhibiteur de déacétylases d'histone (HDACi).

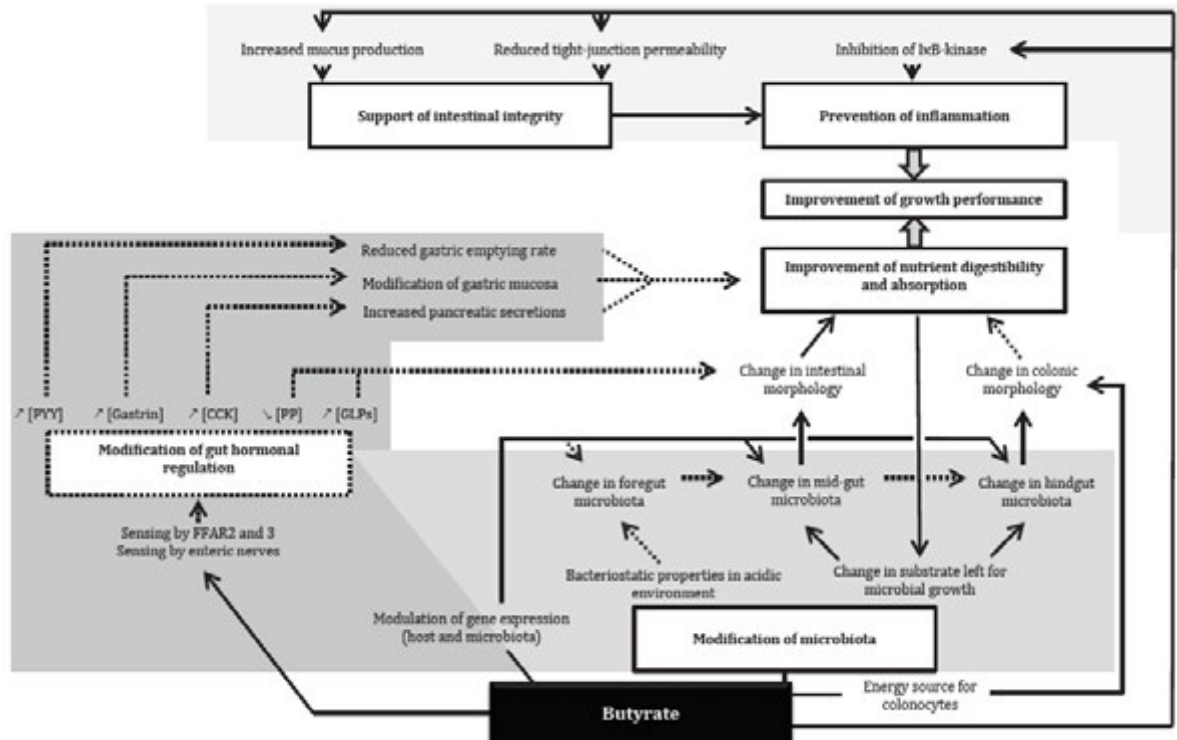
En plus d'offrir un survol de la gamme étendue des processus de signaux que le butyrate peut moduler, je présenterai des données démontrant que les effets de la supplémentation en butyrate diététique au bétail dépendent fortement de l'emplacement entérique où le butyrate ingéré est administré dans le tractus digestif des animaux. Par exemple, au moins dans certaines conditions, des concentrations élevées de butyrate dans l'intestin antérieur et moyen des poulets à griller peuvent induire des effets négatifs sur la diversité et/ou l'inflammation du microbiote cæcal, par rapport à des concentrations plus élevées de butyrate dans l'intestin postérieur.

Il est donc d'une importance critique d'investiguer la cinétique de la distribution du butyrate pour différents produits de butyrate commerciaux, pour expliquer les effets distincts qu'ils peuvent avoir sur la santé et sur le rendement des animaux d'élevage.

## **Introduction to butyrate**

In the lower intestinal tract of monogastrics, short-chain fatty acids are produced in the intestinal lumen by bacterial fermentation of dietary fibers and resistant starches. Among the most prevalent ones (acetic acid, propionic acid and butyric acid), butyric acid/butyrate has received special attention for its beneficial effects in humans and production animals. In the field of human medicine, butyrate is studied for its role in maintaining energy metabolism and intestinal homeostasis, in both normal and disease contexts, including cancer, inflammatory diseases and metabolic syndrome. In livestock animals, investigating butyrate's working mechanisms is mainly related to its effects on gut development and animal health and performance.

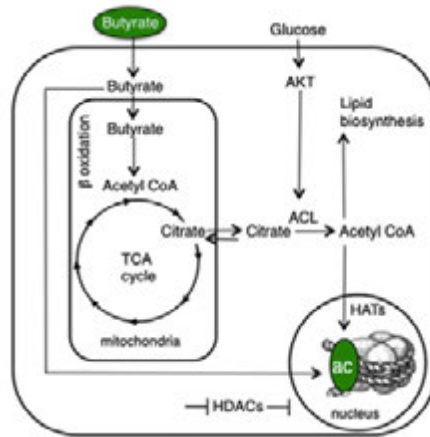
Butyrate can directly or indirectly elicit a wide range of cell biological pathways, reflecting its capacity to modulate physiological signaling transduction pathways in different ways (Figure 1).



**Figure 1:** Examples of butyrate-induced effects on the endocrine system, immune response, intestinal integrity and microbiota composition that might contribute to improved animal performance. From (Moquet, 2018).

## Butyrate and epigenetic effects

In eukaryotic cells, all the different biological responses modulated by butyrate can be brought back to its ability to instigate cellular effects, either as a source of energy, as a ligand to G-coupled protein receptors, or by modulating gene expression via epigenetic effects. Butyrate is a member of a well-studied class of epigenetic substances known as histone deacetylase inhibitors (HDACi) (Berni Canani et al., 2012), as has also been described to stimulate the activity of histone acetyltransferase (HAT) (Donohoe et al., 2012) (Figure 2). These molecules promote the acetylation of proteins linked to DNA, thereby altering chromatin structures and gene expression patterns.



**Figure 2:** Model for butyrate-induced histone acetylation mechanisms. From (Donohoe et al., 2012).

Both in humans as in animals, there is a growing interest in inducing epigenetic changes via dietary compounds such as butyrate, as they have been shown promise trials aiming at mitigating different disease states (Sossai, 2012). For production animals, the quest for alternatives to the use of antibiotics as growth promoters, has been an important driver to explore the potential of butyrate as gut health promoting feed supplement (Huyghebaert et al., 2011).

However, the effects that are elicited by dietary butyrate supplementation have been described to be variable (Moquet et al., 2018). This might come as no surprise: the enormous complexity of mechanisms that may be modulated by (exogenous) butyrate makes it difficult to predict which of these have the potential to have a profound impact to underlie improvements in animal performance, and to which extent, and in which production conditions. In addition, it is critical to note that endogenous butyrate production is located in the hindgut of monogastric production animals, while most dietary (exogenous) butyrate will (also) deliver butyrate more proximally in the digestive tract. Moreover, different commercially available butyrate products have distinct enteric butyrate release profiles (Moquet et al., 2016). We therefore hypothesized that at least a part of this variation could be brought back to differences in the regions where butyrate was delivered in the gastro-intestinal tract (GIT).

### Site-specific effects of butyrate

In an experiment where broilers were challenged by a diet high in rapeseed meal, birds were given several butyrate supplements, each differing in the GIT-region where they delivered the butyrate (Moquet, 2018). Surprisingly, a higher butyrate presence in the proximal GIT, as shown in the unprotected butyrate fed group, was associated with shifts in microbial composition, a reduction of hindgut microbial diversity, and an increase of immunity-associated gene expression in the intestine, all of which are hallmarks of dysbiosis (Table 1). A similar inflammatory signature was observed when butyrate concentrations were elevated in the mid-gut, although no sign of bacterial hindgut dysbiosis was detected in these birds. When butyrate levels were increased in the hindgut as well, as in the birds fed precision-delivery protected butyrate (ADIMIX®Precision), a longer intestinal feed retention time and a tendency for FCR improvement and Met digestibility could be observed, without negative effects on caecal microbiota composition or inflammatory status (Table 1) (Moquet, 2018).

**Table 1** Overview of some effects associated with delivery of exogenous butyrate in the fore-, mid- and hindgut, as observed in the experimental model described in (Moquet, 2018).

	relatively high levels of exogenous butyrate in		
	stomach	mid-gut	hind-gut
apparent ileal dig.	↓ (trend) for Ile, Leu, Phe, His, Lys		↑ (trend) for Met and His
inflammatory gene expression	↑ in ileum and colon	↑ in duodenum, ileum and colon	no
signs of caecal dysbiosis	yes	no	no

## Summary

Butyrate is a short-chain fatty acid with the potential to trigger (epigenetic) changes in gene expression and to induce many biological responses along the gastro-intestinal tract and in peripheral tissues. Although most research studies emphasize the beneficial properties of this molecule, we argue that the effects of supplementing exogenous butyrate can vary greatly, depending on where butyrate is released in the digestive tract. At least in some conditions, butyrate products might profoundly distinct effects on responses related to gut health, which has important consequences on its application as an animal performance promoting feed additive.

## References

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**Sossai, P.** (2012). Butyric acid: what is the future for this old substance? *Swiss medical weekly* **142**, w13596.

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## **The Effects of Diet and Epigenetic Alterations on the Gut Microbiome, Inflammation and Poultry Production**

### **Effets des altérations diététiques et épigénétiques sur le microbiome de l'intestin, sur l'inflammation et sur la production de volaille**

*Michael H. Kogut*

*Southern Plains Agricultural Research Center, USDA-ARS College Station TX 77845*

*Mike.kogut@usda.gov*

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#### **Abstract**

The gut microbiota is a fundamental force influencing diverse aspects of avian physiology. Microbiome studies are at a critical juncture and facing a challenging transition from descriptive studies of association towards mechanistic studies tackling causality. Essential for this transition is a diversity of thinking (chemical and systems biology, metabolism, microbiology, physiology and immunology) and approaches (assays and models). The gut microbiota plays an integral role in digesting food, harvesting energy and regulating immune development. In particular, it can generate numerous bioactive compounds, including short chain fatty acids (SCFAs), choline metabolites and lipids, that are important to host physiology. Microbial metabolites are critical messengers in the crosstalk between microbiome and host cells inducing not only local effects in the gut, but also changes in distant organs. Further, these low-molecular-weight compounds and nutrients actively participate in various epigenomic mechanisms that reprogram the genome by altering the transcriptional machinery of a cell in response to environmental stimuli. These epigenetic modifications are caused by a set of highly dynamic enzymes, notably histone acetylases, deacetylases, DNA methylases, and demethylases, that are influenced by microbial metabolites and other environmental cues. Host expression of histone acetylases and histone deacetylases is important for regulating communication between the intestinal microbiota and the host cells. Histone acetylases and deacetylases influence the molecular expression of genes that affect not only physiological functions but also behavioral shifts that occur via neuroepigenetic modifications of genes. The underlying molecular mechanisms, however, have yet to be fully elucidated and thus provide a new area of research.

#### **Résumé**

Le microbiote intestinal est une force fondamentale qui influence divers aspects de la physiologie aviaire. Les études du microbiome sont à un point déterminant critique où l'on doit passer des études descriptives d'association aux études mécanistes s'attaquant à la causalité. L'essentiel pour cette transition repose sur une diversité de pensées (biologie des éléments chimiques et des systèmes, métabolisme, microbiologie, physiologie et immunologie) et de méthodes (biotests et modèles). Le microbiote intestinal joue un rôle intégral pour digérer les aliments, emmagasiner l'énergie et réguler le développement humanitaire. En particulier, il peut produire plusieurs composés bioactifs, dont les acides gras à chaîne courte (AGCC), les métabolites de la choline et les lipides,

qui sont importants pour la physiologie de l'hôte. Les métabolites microbiens sont des messagers critiques dans le dialogue entre le microbiome et les cellules hôtes, induisant non seulement des effets dans l'intestin, mais aussi des changements dans des organes éloignés. De plus, ces composés et nutriments à faible poids moléculaire participent activement à divers mécanismes épigénomiques qui reprogramment le génome, en altérant la mécanique de transcription d'une cellule en réponse aux stimuli de l'environnement. Ces modifications épigénétiques sont causées par une série d'enzymes hautement dynamiques, notamment les acétylases et déacétylases d'histone, les méthylases et déméthylases d'ADN, qui sont influencées par les métabolites microbiens et par d'autres signaux environnementaux. L'expression des acétylases et déacétylases d'histone chez le sujet est importante pour réguler la communication entre le microbiote intestinal et les cellules hôtes. Les acétylases et déacétylases d'histone influencent l'expression moléculaire des gènes qui affectent non seulement les fonctions physiologiques, mais aussi les changements comportementaux survenant suite aux modifications neuroépigénétiques des gènes. Les mécanismes moléculaires sous-jacents, toutefois, restent encore à élucider complètement, ce qui ouvre un nouveau champ de recherche.

## **Introduction**

The gastrointestinal tract, or “gut”, regulates homeostasis of the microbiological, physiological, and physical functions that allows the host to endure infectious and non-infectious stressors that it encounters (Crhanova et al., 2011, Sansonetti, 2004, Maslowski and Mackay, 2010, Quintero-Fiho et al., 2012). Because the gut has the greatest surface area separating the environmentally exposed lumen and the internal subepithelial tissue, it is constantly exposed to infectious and non-infectious stressors making it an active immune organ containing more resident immune cells than any other organ in the host. The gut mucosal immune system, a highly-regulated network of innate and acquired elements, provides a remarkable ability to respond and modify to these extremely diverse encounters (Thaiss et al., 2016; Honda and Littman, 2016). The development of the different divisions of the immune response has corresponded with the acquisition and maintenance of a symbiotic microbiota. The microbiota trains, stimulates, and functionally adjusts the different features of the immune system (Hooper and Macpherson, 2010; Hooper et al., 2012).

## **Intestinal Immunity**

Like the systemic immune system, the mucosal immune system is made up of a network of innate and acquired elements. However, unlike the systemic immune system, the intestinal immune system has two distinct functions: the ability to respond to pathobionts (potential pathogenic microbes), invasive pathogens, and microbial products while also maintaining a state of tolerance to the diverse and beneficial commensal intestinal microbes (Broom and Kogut, 2018). Both systems working together through innate immune sensing using pattern recognition receptors (PRRs) on epithelial cells and professional immune cells in the lamina propria (dendritic cells and macrophages), trigger immune pathways resulting in microbial killing and the activation of various acquired immune effector T cells (Th1, Th2, Th17, Treg) all while keeping the resident microbiota in check without generating an overt inflammatory response.

The intestinal innate defenses are characterized by a ‘mucosal firewall’, a system of barriers that separates the luminal side of the intestine from the subepithelial tissues (Macpherson et al.,

2009; Belkaid and Hand, 2014). The reliability of the mucosal firewall is vital for the interactions between the immune system components and the intestinal contents. The first component of the mucosal firewall is the microbiological barrier where the microbiota live in or at the upper mucus layer. These commensal bacteria function to provide colonization resistance against pathogen colonization, produce metabolites/components that modulate immune signaling, and promote immune homeostasis (Garrett et al., 2010, Belkaid and Hand, 2014; Belkaid and Harrison, 2017). The second firewall is the chemical barrier consisting of the mucus overlaying the gut epithelium. The mucus regulates contact between the commensal bacteria and the epithelial cells. This division between the epithelium and commensals is achieved by the activity of the mucus produced by goblet cells in the epithelium, antimicrobial peptides released by the epithelial cells, and mucosal IgA produced by dendritic cells in the intestine (Vaishnava et al., 2011, Belkaid and Harrison, 2017). The third component of the firewall is the physical barrier provided by the single cell epithelial cell layer. The intestinal epithelium is a single cell layer that assists the absorption of nutrients while providing a physical barrier that prevents both pathogen invasion and extra-intestinal translocation of commensal microbes. Besides being the primary barrier preventing a microbial breach of the intestine, the epithelial cells should also be considered part of the cellular component of the innate immune response possessing PRRs for sensing microbial-associated molecular patterns (MAMPS), but also capable of producing cytokines and chemokines to drive an inflammatory response against pathogen infection. The final component of the mucosal firewall is the immunological barrier where the professional immune cells (macrophages, dendritic cells, lymphocytes) reside in the lamina propria (Abraham and Medzhitov, 2011). Further innate sensing of microbes is conducted by the macrophages and dendritic cells which can present antigens to T cells resulting in the differentiation and activation of various T cell subsets (Th1, Th2, Th17 or Treg) (Abraham and Medzhitov, 2011). Specialized epithelial cells of the gastrointestinal tract function together with lymphoid, myeloid, and stromal cells to secrete mucus, antimicrobial peptides, IgA, and chemokines that limit direct contact between the epithelium and infectious agents and activate target cells that mediate innate defenses (Medzhitov, 2001; Akira et al., 2006; Abreu et al. 2005; Kawai and Akira, 2009; Mantis and Forbes, 2010). The importance of these epithelial defense mechanisms is highlighted by the ability of enteric pathogens to target these mechanisms to achieve invasion and dissemination (Awad et al., 2017; Lu and Walker, 2001; Fischback et al., 2006; Goto et al., 2017; Alemka et al., 2012).

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## **Microbiota interactions with immune system**

The host-microbiota interaction that affects the host metabolism, immunity and health is exceedingly complex (Marchesi et al., 2016). This crosstalk is mediated by dietary nutrients, host and microbiota metabolites, microbial structural components, as well as antimicrobial compounds. Microbiota growth and anatomical location are regulated by the host through production of non-specific antimicrobial peptides such as defensins (Xiao et al., 2006; Bomnineni et al., 2014), IgA (Lammers et al., 2010; Den Hartog et al., 2016), and miRNAs that regulate bacterial transcripts and bacterial growth (Liu et al., 2016).

The commensal microbes in the intestinal tract sense the local environment to induce biochemical pathways to activate bacterial metabolism that allows them to avoid, alter, and/or survive host innate immune killing. Furthermore, some microbial-based molecules can promote specific commensal processes that are beneficial to both host and microbe. Similarly, the host detects the microbes through their production of specific molecules or components with unique molecular patterns that leads to activation of innate and acquired immune responses. Thus, the adaptation of the commensal bacteria (as well as viruses and fungi) living in the intestine of a host has resulted in a mutually beneficial coexistence for both microbiota and host during homeostasis (Kogut, 2013; Kogut et al., 2017; Broom and Kogut, 2018). The interdependent relationship between host and microbiota pointedly influences the host immune response to induce an immune tolerance to commensal microbes while also maintaining responsiveness to invading pathogens (Bene et al., 2017; Guo et al., 2017; Shi et al., 2017). Altering the intestinal microbial communities disturbs this immune balance and leads to immune dysregulation and susceptibility to diseases.

Sensing of the microbiota by PRRs generates a number of mechanisms that promote the host-microbiota relationship while preventing infection by pathogenic organisms. Microbial signals induce pro-inflammatory cytokines such as IL-23 and IL-1 $\beta$  from macrophages and DCs that then activate IL-17 and IL-22 production by T cells, leading to the production of steady-state physiological inflammation (Kogut et al., 2018). DCs can carry microbiota antigens to the Peyer's patches and/or small lymphoid follicles in the avian intestine, where they drive the differentiation of regulatory T cells (Tregs) and Th17 T cells that, in turn, induce the differentiation of IgA-producing plasma B cells that secrete further amounts of IgA.

## **Microbiota-based metabolites and immunity**

The microbiota is directly engaged in maintaining the functional innate immunity of the host. The host immune system consistently senses the intestinal microenvironment to determine the metabolic state and colonization status (Levy et al., 2016). In the steady state, the metabolites and/or components of the commensal microbiota are recognized by various PRRs, including toll-like receptors (TLRs) and Nod-like receptors (NLRs), to regulate intestinal epithelial barrier function, cellular lifespan of phagocytes, and induce secretion of antimicrobial peptides and IgA (Levy et al., 2016; Blacher et al., 2017). Furthermore, beneficial bacteria ferment dietary fibers to produce small chain fatty acids (SCFA) which stimulate the production of anti-inflammatory cytokines (Levy et al., 2016; Blacher et al., 2017) that drives the production of regulatory T cells (Tregs). In addition, the microbiota influences the priming signal of the inflammasome activation that leads to the transcription of IL-6, as well as pro-IL-1 $\beta$  and pro-IL-18. The gut microbiota is involved

in maintaining intestinal immune homeostasis by stimulating different arms of the T-cell response. Segmented filamentous bacteria (SFB) are potent promoters of Th17 cells in the intestine; whereas, polysaccharide A from the commensal *Bacteroides fragilis* stimulates the generation of Tregs (Levy et al., 2017). Alternatively, pattern recognition by TLRs and NLRs can also induce the maintenance of tolerance (Levy et al., 2017).

Lastly, it has become readily apparent that the intestinal immune system can also detect the metabolic state of the microbiota by recognition of microbial metabolites via their PRRs (Levy et al., 2017; Blacher et al., 2017). The microbiota, using a number of biochemical pathways, metabolizes both diet- and host-derived metabolites that then influence various components of the intestinal immune system. For example, the microbiota converts non-digestible fibers to SCFA that have a number of anti-inflammatory activities (Postler and Ghosh, 2017). Dietary tryptophan can be degraded by the microbiota into indoles which promote epithelial cell barrier function (Postler and Ghosh, 2017). Likewise, the microbiota can metabolize dietary arginine to polyamines that inhibit the production of pro-inflammatory cytokines by macrophages (Postler and Ghosh, 2017). The microbiota converts primary host-derived hepatic bile acids to secondary bile acids that inhibit pro-inflammatory cytokine secretion by DCs and macrophages (Thaiss et al. 2014). Besides having a repertoire of metabolite sensing receptors, the host has developed immune signaling pathways (inflammasomes) expressed in various intestinal cell subsets (macrophages, DCs, epithelial cells, T cells) that recognize microbial-mediated metabolic activity that can stimulate anti-microbial activity involved in stable colonization of the intestine (Levy et al., 2015; Wang et al., 2015; Birchenough et al., 2015).

Therefore, there is intimate cross-talk between the microbiota and the host that is steered by metabolite secretion and immune signaling that has critical influence in animal health and disease through multiple physiological functions of the host.

## Colonization Resistance

The commensal bacteria also provide protection to the host from colonization by exogenous pathogens by a process known as colonization resistance (van der Waaij et al, 1971 Buffie and Pamer, 2013, Rangan and Hang, 2017). Two primary mechanisms of colonization resistance have been identified: direct, where the microbiota are in direct competition against pathogen colonization and indirect, where the commensal microbiota stimulate the innate and acquired immune systems as described in the previous sections.

The direct competition of colonization resistance involves multiple processes that include: (a) *occupying microbial niches*: specific commensal microbes can prevent pathogen colonization to the intestinal mucosa by occupying the niche where a pathogen would normally establish (Belkaid and Hand, 2014, Sassone-Corsi and Raffatellu, 2015); (b) *limiting carbon sources*: individual commensals, such as *Bacteroides thetaiotaomicron* can metabolize fucose (sugar) molecules thereby preventing the availability of this sugar moiety in to certain pathogens in the intestine (Belkaid and Hand, 2014, Sassone-Corsi and Raffatellu, 2015); (c) *Siderophore production*: some commensals possess the genes for the production and acquisition of the metal ion iron via iron chelators (siderophores) that can uptake iron limiting its availability to pathogens, especially during gut inflammation (Belkaid and Hand, 2014, Deriu et al., 2013); (d) *Production of antimicrobial*

*compounds*: some Enterobacteriaceae commensals produce antimicrobial compounds, such as bacteriocins, that target competitor pathogens (Chassaing and Cascales, 2018); (e) *Contact-dependent delivery of toxins*: some commensals can express a type VI secretion system (T6SS), a needle-like injection system that inject toxic proteins into close competitors in a contact-dependent manner (Pezoa et al., 2016, Sana et al., 2016, Chassaing and Cascales, 2018).

The indirect mechanisms of colonization resistance against enteric pathogens are mediated by microbiota-stimulated activation of both host innate and acquired immunity (Belkaid and Hand, 2014, Sassone-Corsi and Raffatellu, 2015, Rangan and Hang, 2017). Commensal bacteria can indirectly control pathogen colonization by stimulating of intestinal barrier function and innate immunity as described above. In this case, the commensal bacteria, through the production of metabolites or release of surface components (LPS, peptidoglycans, DNA, etc.) are recognized by the PRRs on the intestinal epithelial and professional immune cells that result in the production and secretion of mucins, secretory IgA (sIgA), and antimicrobial peptides, all of which either increase barrier function of the mucosal firewall or are lethal to pathogens (Belkaid and Hand, 2014, Sassone-Corsi and Raffatellu, 2015). Furthermore, the commensal microbiota can enhance epithelial barrier function by producing small chain fatty acids (SCFA), such as butyrate, from dietary fibers (Guilloteau et al. 2010). T-cell subsets in the intestinal lamina propria are involved in the establishment and the maintenance of colonization resistance. A balanced Thelper/Tregulatory status is generated by diverse populations of commensal bacteria in the intestine. For example, segmented filamentous bacteria promote acquired immunity by T cells by stimulating Th17 cells whereas other commensals, such as *Clostridium* and *Bacteroides fragilis*, induce the expansion of T regulatory cells that can regulate inflammatory responses through the production of IL-10 (Lee and Mazmanian, 2010, Round and Mazmanian, 2009, O'Mahony et al., 2008, Ivanov et al., 2009).

## **Gut Microbiota as an Epigenetic Regulator of Gut Function**

Epigenetics involves genomic modifications through post-translational and post-transcriptional modification induced by environmental factors, but without modifying the nucleotide sequence of the host cell (Shenderov, 2012). Epigenetic mechanisms regulate transcriptional control by external environmental cues such as diet, stress events, disease, infections, and host-microbe cross-talk (Shenderov, 2012, Chen et al., 2017, Woo and Alenghat, 2017, Grabiec and Potempa, 2018). Since epigenetic events do not alter the DNA, the epigenomic effects are associated with the attachment of different chemical groups to DNA, histones, and chromatin post-translationally and the epigenetic alterations can persist for several generations (Furrow et al., 2011, Shenderov, 2012). These epigenetic alterations affect both the chromatin structure and serve as recognition elements for proteins with motifs dedicated to binding particular modifications

Since the gut microbiota plays such a pivotal role in poultry metabolism, microbiota-induced epigenetic alterations by dietary nutrients could be a significant environmental factor affecting poultry performance and health. Based on studies in mammals, microbiota-generated metabolites of dietary components can be epigenetic activators of gene expression that modify or inhibit enzymes involved in epigenetic pathways (Alenghat et al., 2013, Alenghat and Artis, 2014, Hullar et al., 2014, Woo and Alenghat, 2017). This can best be exemplified by the production of SCFA (acetate, propionate, butyrate) produced by intestinal microbiota by bacterial fermentation of non-digestible carbohydrates (Hu and Guo, 2007, Guilloteau et al. 2010, Jiang et al., 2015). Butyrate is

best known for its beneficial effects on intestinal barrier function, anti-inflammatory activity, and as the primary source of energy to intestinal epithelial cells (Hamer et al., 2008, Guilloteau et al. 2010, Leonel and Alvarez-Leite, 2012, Huang et al., 2015). Butyrate regulates these biological activities of host gut health by functioning as a histone deacetylase inhibitor (HDAC) (Canani et al., 2012, Liu et al., 2018). Butyrate anti-inflammatory activity is mediated by HDAC suppression of NF- $\kappa$ B in phagocytic cells and dendritic cells, increased production of anti-inflammatory cytokines, and the increased differentiation of naive T cells into T regulatory cells (Usami et al., 2008, Arpaia et al., 2012, Smith et al., 2013). Other microbial metabolites derived from dietary components, such as nicotinamide adenine dinucleotide (NAD)-dependent deacetylases called sirtuins, have been shown to mediate the regulation of epigenetic modifications, including DNA methylation noncoding RNAs and histone modification, in the host intestinal immune-barrier function of mammals (Kobayashi et al., 2012, Ganai et al., 2012, Singh et al., 2012). Further research is need to determine whether such gut microbiota metabolite-mediated epigenetic modifications of the immuno-barrier function occur in the poultry intestine.

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## Transition Cow Nutrition: Setting Her Up for Success

### Nutrition de la vache en période de transition : Assurer sa réussite

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#### Abstract

The early lactation cow is challenged to produce copious amounts of milk, fight infection, and remain fertile even though energy intake is inadequate. The longevity of the dairy cow is linked to her ability to transition from gestation to lactation without clinical complications. She relies on maternal adaptations including elevated adipose tissue lipolysis, peripheral fatty acid (FA) oxidation and hepatic gluconeogenesis, as well as reductions in peripheral insulin action and augmented ketogenesis that work to spare glucose for milk synthesis. Unfortunately, hepatic injury in the form of triglyceride deposition, inflammation, and activation of the acute-phase response may develop. Lipidomic data suggests that the glycerophospholipid phosphatidylcholine and the sphingolipid ceramide are targets to control hepatic triglyceride disposal and insulin action, respectively. Limited phosphatidylcholine synthesis may prevent hepatic lipoprotein triglyceride secretion, and ceramide accrual may provoke insulin antagonism and lipolysis. Optimized methyl donor and FA nutrition has potential to maximize phosphatidylcholine synthesis and suppress ceramide production to minimize hepatic steatosis and inflammatory insult. Dietary supplementation of one-carbon/methyl donors including choline, methionine, betaine, and serine are promising therapies to support phosphatidylcholine synthesis and bolster transition cow health. Science has also centered on the beneficial and adverse outcomes associated with FA supplementation. Palmitic acid feeding induces ceramide production and thus challenges periparturient well-being. Alternatively, dietary rumen-protected docosahexaenoic acid may improve insulin sensitivity and optimize the ability of methyl donors to stimulate hepatic phosphatidylcholine synthesis. The interplay between methyl donor and FA nutrition should be considered to prepare the transition cow for a successful lactation.

#### Résumé

La vache en début de lactation doit produire de grandes quantités de lait, combattre les infections et demeurer fertile, même si son apport énergétique est inadéquat. La longévité de la vache laitière dépend de sa capacité à faire la transition entre gestation et lactation sans complications cliniques. Elle compte sur les adaptations maternelles, incluant la lipolyse élevée des tissus adipeux, l'oxydation des acides gras (AG) périphériques et la gluconéogenèse hépatique, de même que sur les réductions de l'action de l'insuline périphérique et sur l'augmentation du cétogénèse, qui aident à conserver le glucose pour la synthèse du lait. Malheureusement, une blessure du foie peut se développer suite au dépôt de triglycérides, à l'inflammation et à l'activation de la réponse en phase aiguë. Les données lipidomiques donnent à penser que la lécithine du glycérophospholipide et le

céramide du sphingolipide sont des cibles pour contrôler l'élimination des triglycérides hépatiques et l'action de l'insuline, respectivement. La synthèse limitée de la lécithine peut prévenir la sécrétion de triglycérides lipoprotéiques hépatiques, alors que l'accumulation de céramide peut provoquer l'antagonisme à l'insuline et la lipolyse. Le donneur méthyle optimisé et la nutrition aux AG a le potentiel de maximiser la synthèse de la lécithine et de stopper la production de céramide pour minimiser la stéatose hépatique et l'insulte inflammatoire. La supplémentation diététique des carbonnes/donneurs méthyle, incluant la choline, la L-méthionine, la bétaine – et possiblement la sérine – sont des thérapies prometteuses pour favoriser la synthèse de la lécithine et renforcer la santé de la vache en période de transition. Concernant la nutrition aux AG, la supplémentation d'acide palmitique induit la production de céramide, ce qui peut inhiber la sensibilité à l'insuline pour accélérer la mobilisation du gras et influencer la santé. En revanche, l'acide docosahexaénoïque diététique qui protège la panse peut améliorer la sensibilité à l'insuline et optimiser la capacité des donneurs méthyle de stimuler la synthèse de la lécithine hépatique. On devrait prendre en considération l'interaction entre le donneur méthyle et la nutrition aux AG pour préparer la vache en période de transition à une bonne lactation.

## **Introduction**

The long-term longevity of high-production, fertile, and healthy dairy cows is desirable. Unfortunately, Holstein cows typically achieve less than three parities before culling (Hare et al., 2006). Grandl and coworkers (2016) emphasize that elevated milk yield may persist for five to eight lactations; therefore, the true productive lifespan of cows is lost. Enhancing longevity of high-performing cows deserves consideration because of current environmental and industry sustainability concerns in the reality of climate change. Undeniably, survival rates are influenced by breed, management and economic factors such as milk price and feed costs as well as the availability of replacement heifers. However, carefully considered dairy cattle nutrition holds much potential to support a healthy and productive life for the cow. This is of particular importance for the periparturient dairy cow that undergoes dynamic changes in physiology and diet. This review will focus on the ability of methyl donor and fatty acid (FA) nutrition to influence hepatic and systemic health of the transition cow.

## **Hepatic Impairment in Transition Dairy Cows**

Considerable effort has helped define transition cow biology and maladaptations that provoke metabolic dysfunction characterized by hyperlipidemia (elevated blood free FA), fatty liver disease (FLD), and rampant ketone production (Drackley, 1999; Ingvarsen, 2006). Specifically, the increase in energy demand of lactation coupled with inadequate energy intake drives body fat mobilization (i.e., lipolysis). This condition is exacerbated by elevated body condition prepartum (i.e., body fat mass) and reductions in insulin sensitivity. Notably, peripartal changes in the plasma FA profile closely mimics fat composition with elevations in saturated palmitic acid (C16:0) and monounsaturated oleic acid (C18:1; Douglas et al., 2007). On the other hand, the proportion of polyunsaturated FA such as linolenic acid (C18:3) and eicosatetraenoic acid (C20:4) decrease in plasma as parturition approaches. The lipolytic surge of circulating FA with a high-degree of saturation enter the liver for catabolic mitochondrial  $\beta$ -oxidation or anabolic re-esterification in the form of triglycerides (TG). With the assistance of microsomal TG transfer protein, hepatic TG

are packaged within very low density lipoproteins (VLDL) for secretion. However, the transition cow experiences incomplete  $\beta$ -oxidation and a limited capacity to secrete TG within VLDL, which are predisposing factors for hepatic ketogenesis and excessive TG accumulation (Pullen et al., 1990; Litherland et al., 2011). Severe TG deposition promotes FLD which compromises fertility and milk production performance within the lactation if not resolved (Bobe et al., 2004). Recent findings have also revealed that FLD develops with inflammation, activation of the acute-phase response, and oxidative stress that further challenge the health and well-being of the cow (Ametaj et al., 2005; Sordillo and Aitken, 2009).

With regard to longevity, Bobe and colleagues (2004) recognize that a greater proportion of older cows have fatty liver which is likely attributed to greater body fat at calving, heightened milk production, and longer calving intervals. To achieve gains in long-term longevity, refined dietary practices that enhance insulin sensitivity, minimize fat mobilization and promote hepatic TG disposal are required. We recognize that these periparturient solutions, some of which that are described below, may direct nutrient partitioning to body fat reserves; however, controlled lipolysis will preserve health for a successful transition. Thereafter, properly timed feeding strategies that reinforce homeorhetic control will allow the cow to achieve her maximum milk production potential. The current focus on methyl donor and FA nutrition is warranted because of their ability to influence hepatic TG disposal and inflammation, systemic oxidative stress and insulin sensitivity, and lipolysis.

### **Fatty Acid Nutrition to Control Nutrient Partitioning**

The supplementation of fats and oils to transition cow diets is considered an approach to enhance the energy density of the diet to support the energetic demands of milk production. Such practice is warranted over increasing dietary starch content, which may compromise rumen health, trigger endotoxemia, and potentially exacerbate liver injury. However, research suggests that the FA composition of supplemental fat may uniquely modify energy metabolism, glucose economy, health, lactation, and reproduction even if energetically equivalent.

Omega-3 (n-3) FA such as  $\alpha$ -linolenic acid (ALA; C18:3n-3), eicosapentaenoic acid (EPA; C20:5n-3), and docosahexaenoic acid (DHA; C22:6n-3) have been generally recognized as “health-promoting” FA in humans. Omega-3 FA appear to elicit anti-inflammatory and insulin-sensitizing properties, reverse FLD, reduce cardiovascular morbidity, and prevent premature mortality in non-ruminants (Calder and Yaqoob, 2009; Nobili et al., 2011). A growing body of literature suggests that omega-3 FA feeding during transition may also be a means to support health and performance in dairy cows. For example, feedstuffs of the flax variety are rich in ALA and have been studied extensively in cows. Feeding extruded flaxseed during the transition period increased early lactation milk production, maintained postpartum body weight, and enhanced energy balance, relative to an unsupplemented control diet (Zachut et al., 2010). Feeding multiparous periparturient cows diets containing whole flaxseed kept postpartum plasma total FA and  $\beta$ -hydroxybutyrate concentrations low, whereas feeding a fat supplement rich in saturated palmitic and stearic acids robustly elevated these markers of poor metabolic health (Petit et al., 2007). Not surprisingly, whole flaxseed supplementation also decreased postpartum TG levels and increased glycogen concentrations in liver, relative to saturated fat feeding. Indeed, cows intravenously infused linseed oil (i.e., flax) experienced lower plasma total FA and  $\beta$ -hydroxybutyrate levels, and liver TG content, relative

to cows infused a saturated tallow emulsion (Mashek et al., 2005). Interestingly, these authors hypothesized that specific A may uniquely modulate adipose tissue metabolism (e.g., lipolysis).

Although the beneficial effects of omega-3 FA feeding on health and production may be attributed to enhanced dry matter intake in some instances, this outcome is not always observed (Gonthier et al., 2005; Zachut et al., 2010). An alternative consideration is that the intestinal absorption of omega-3 polyunsaturated FA may increase adipose tissue insulin sensitivity to suppress lipolysis. The mechanistic ability of omega-3 FA to enhance insulin sensitivity likely involves their ability to enhance FA oxidation, reduce sterol-regulatory element binding protein-mediated lipogenesis, and suppress inflammation (Kalupahana et al., 2011). In fed dairy cows, the abomasal infusion of ALA-containing linseed oil had an insulin-sensitizing effect compared to tallow infusion (Pires et al., 2008). Moreover, linseed oil infusion caused a greater reduction in total FA disappearance following a glucose challenge in Holstein cows (Pires et al., 2008). These results suggest that ALA may enhance the anti-lipolytic actions of insulin. Albeit in steers, the abomasal infusion of menhaden oil rich in omega-3 FA was compared to a mixed oil containing oleic and linoleic acids (Gingras et al., 2007). Indicative of enhanced insulin signaling, the abomasal delivery of long-chain omega-3 FA increased protein kinase B phosphorylation (indicative of enhanced insulin signaling) and glucose transporter-4 protein abundance in skeletal muscle. Insulin-mediated glucose disposal was also enhanced by menhaden oil treatment. The ability of omega-3 FA to promote insulin action may also involve elevations in plasma insulin-like growth factor-1 concentrations (Childs et al., 2008).

The sphingolipid ceramide is considered a key driver of palmitic acid-induced insulin resistance (Summers, 2006). A developing hypothesis in dairy cows is that lipolytic- or diet-derived palmitic acid stimulates ceramide synthesis which in turn amplifies insulin antagonism and nutrient partitioning towards milk synthesis. In dairy cattle, we have shown that ceramide accrual develops in postpartum cows or feed-restricted dry cows experiencing increased plasma total FA levels and hepatic lipid deposition (Rico et al., 2015; Davis et al., 2017a; Rico et al., 2017b). Such outcomes are in positive association with circulating palmitic acid supply and inversely related to glucose-stimulated reductions in total FA (Mathews et al., 2016; Rico et al., 2017a). Moreover, we have shown that high-purity palmitic acid feeding increases circulating ceramide levels in lactating cows, relative to no-added fat, stearic acid, or medium-chain TG supplementation strategies (Rico et al., 2016; Davis et al., 2017b; Rico et al., 2017a). Consistent with our hypothesis, circulating ceramide levels are also consistently positively correlated with milk yield (Rico et al., 2016; Davis et al., 2017b; Rico et al., 2017a). Regarding mechanism, we have shown that ceramide promotes insulin resistance in differentiated primary bovine adipocytes cultured in nutrient excess (Rico et al., 2018). We have also discovered that palmitic acid promotes ceramide production in primary bovine neonatal calf hepatocytes (McFadden et al., 2018). Collectively, our body of work indicates that palmitic acid feeding and the induction of ceramide synthesis may be a means to bolster milk production in low-producing cows beyond peak milk production. Our data also suggests that palmitic acid feeding during early lactation may inadvertently exacerbate body fat mobilization via ceramide-dependent mechanisms that inhibit insulin action. In support, fresh cows fed palmitic acid experienced greater reductions in body weight and body condition score, relative to cows fed a no-added fat diet (de Souza and Lock, 2019). Furthermore, energy partitioning towards the mammary gland for milk synthesis is observed with high palmitic acid supplementation but shifts towards body weight gain when cows are fed a palmitic and oleic acid blend (de Souza et al., 2018).

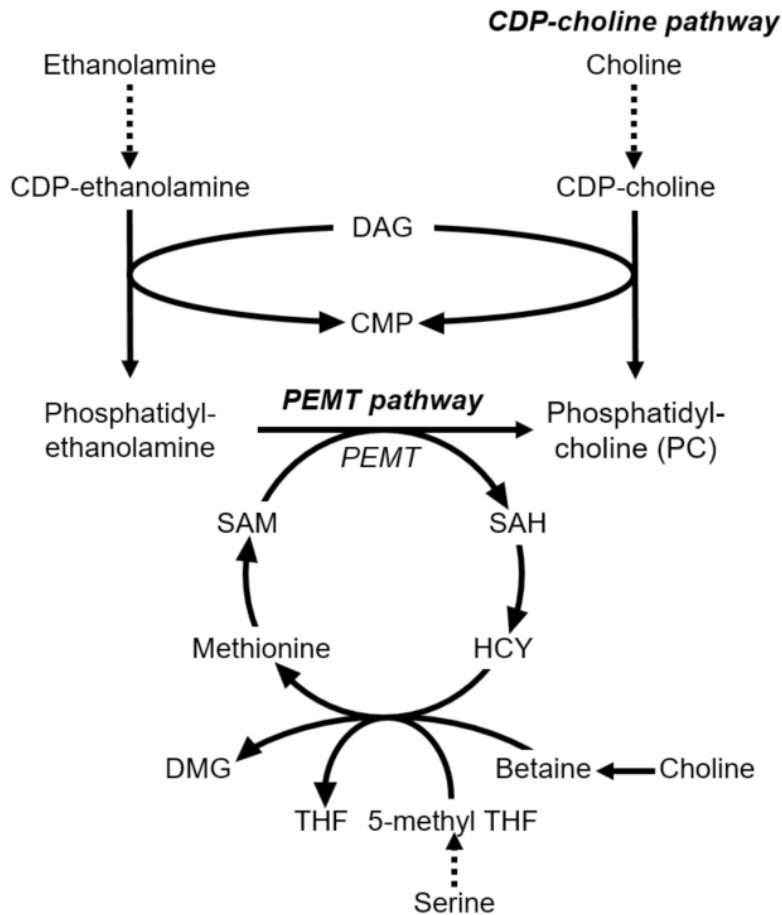
It is possible that saturated and unsaturated FA feeding is necessary to support milk production without provoking uncontrolled body fat mobilization that could impair health. Although care must be taken to avoid severe milk fat depression with unsaturated fat feeding, short-term reductions in milk fat production may be a sacrifice for gains in health, milk production, fertility, and longevity of the cow. Indeed, feeding early lactation cows conjugated linoleic acids has been shown to decrease milk fat content but increase milk production, energy balance, and fertility (Bernal-Santos et al., 2003; Odens et al., 2007; de Veth et al., 2009). Even so, feeding rumen-protected unsaturated fats or saturated/unsaturated fat blends may be a means to avoid concerns pertaining to rumen biohydrogenation and milk fat depression.

### **Strategies to Enhance Hepatic Triglyceride Disposal**

Although limited hepatic apolipoprotein (Apo) B<sub>100</sub> concentrations caused by reduced protein expression may contribute to reduced VLDL assembly and secretion in transition cows (Bernabucci et al., 2004; Bernabucci et al., 2009), an inadequate capacity to synthesize the glycerophospholipid phosphatidylcholine (PC) is generally considered the primary reason for poor TG clearance in cows with FLD. The reason is that PC is the principal lipid constituent of the VLDL monolayer (i.e., the shell that encapsulates the TG) and PC deficiency has been shown to impair VLDL formation and export in rodents (Fast and Vance, 1995). For clarity, the synthesis of PC involves two key pathways: the cytidine diphosphate (CDP)-choline pathway (i.e., the Kennedy pathway) and the phosphatidylethanolamine *N*-methyltransferase (PEMT) pathway (Figure 1). The CDP-choline pathway utilizes choline as the primary polar substrate. Moreover, the CDP-choline pathway prefers diacylglycerol enriched in saturated and monounsaturated FA such as palmitic and oleic acids, respectively (DeLong et al., 1999). In rat primary hepatocytes, ~70% of total PC are produced by the CDP-choline pathway (DeLong et al., 1999). The compensatory PEMT pathway produces the remainder of PC which is functionally relevant in the liver. The PEMT pathway requires phosphatidylethanolamine and donated methyl groups derived from the methylation cycle. Methyl donors including choline, methionine, and betaine, and glycine and serine (one-carbon donors) are involved in *S*-adenosylmethionine (SAM) synthesis and phosphatidylethanolamine methylation. In stark contrast to the CDP-choline pathway, PEMT prefers phosphatidylethanolamine enriched in long- and very-long chain polyunsaturated FA including eicosatetraenoic acid and DHA (DeLong et al., 1999). So much so, that circulating DHA-containing PC has been recognized as a biomarker for PEMT activation in humans (da Costa et al., 2011). Collectively, strong evidence suggests that methyl donor and FA nutrition need to be considered in parallel to optimize PC synthesis and TG secretion in cows.

#### ***Choline and methionine nutrition***

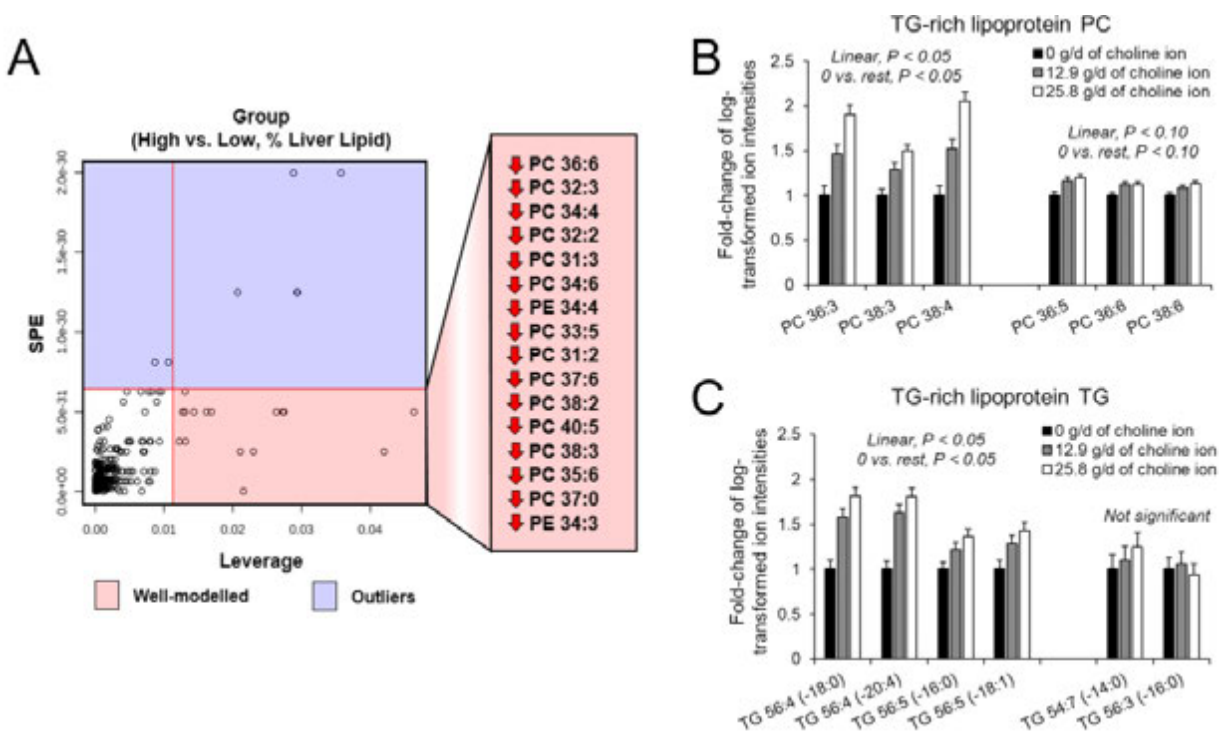
Dietary rumen-protected (RP) choline supplementation is considered an approach to stimulate hepatic PC synthesis and TG disposal in periparturient dairy cows. Nutritionists should consider choline feeding approaches for several reasons. First, choline is rapidly degraded in the rumen if unprotected (Sharma and Erdman, 1989). Second, plasma total choline supply is lowest during early lactation which is less than 10% of levels observed during late lactation (Artegoitia et al., 2014). Third, choline is the principal substrate for the CDP-choline pathway. Fourth, we have



**Figure 1.** Pathways of phosphatidylcholine (PC) synthesis. Abbreviations not described in text: DMG, dimethylglycine; HCY, homocysteine; SAH, S-adenosylhomocysteine; THF, tetrahydrofolate. Dotted lines represents do not include all intermediates.

established that a limited supply of plasma PC that have a high degree of unsaturation is a key feature of FLD in postpartum Holstein cows (Figure 2A). Several studies have demonstrated the TG-lowering abilities of RP choline in dairy cows (Cooke et al., 2007; Zom et al., 2011). More recently, feeding RP choline ions to late gestation cows in negative energy balance has been shown to reduce hepatic TG concentrations in a linear manner (0 to 25.8 g/d of choline ion; Zenobi et al., 2018). This metabolic outcome could be explained by enhanced TG removal. In this same study, we have demonstrated that RP choline ion feeding increases PC and TG in circulating TG-rich lipoproteins that include VLDL (Figure 2B-C). For example, RP choline ion supplementation increases TG-rich lipoprotein PC-36:3, -38:3, and -38:4 during feed restriction; however, the magnitude of the response was diminished for PC that may contain DHA (i.e., PC-36:6 and -38:6; Zenobi et al., 2018). Such findings may suggest that RP choline ion supplementation preferentially activates the CDP-choline pathway to increase the incorporation of FA that are more affiliated with lipolysis. In support, methionine adenosyltransferase 1A (MAT1A) and PEMT protein abundance

was not modified by choline treatment in primary bovine liver cells enriched with hepatocytes (Zhou et al., 2018). The ability of choline to stimulate the CDP-choline pathway and reduce hepatic TG levels may explain observed gains in milk yield with periparturient RP choline chloride feeding (Pinotti et al., 2003). Interestingly, the apparent inability of choline to activate PEMT contrasts observations in women with high choline intake (West et al., 2013).



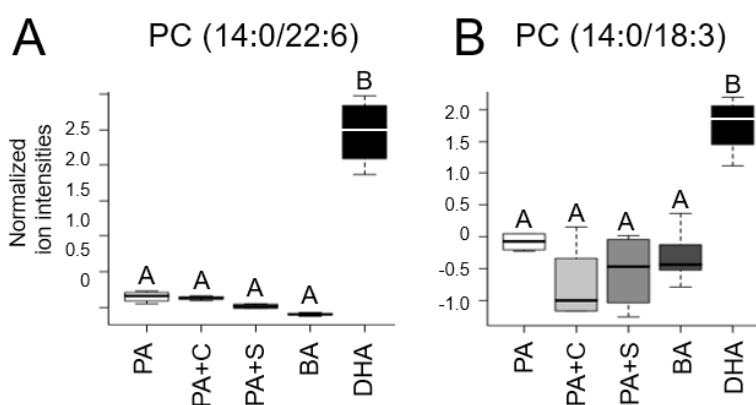
**Figure 2.** Rumen-protected choline supplementation increased lipoprotein PC. (A) Low plasma PC levels are associated with FLD in periparturient Holstein cows. Leverage/squared prediction error (SPE) plot of 301 complex lipids and their relationship with hepatic lipid accumulation. Normalized data represent plasma samples collected from periparturient Holstein dairy cows categorized into low ( $n = 7$ ) or high ( $n = 7$ ) mean (d 5 and 14 postpartum) liver lipid content ( $5 \pm 1$  vs.  $12 \pm 2$  % of wet weight, respectively). Metabolites in lower right quadrant have high loadings and follow the expression pattern of the submodel (i.e., data demonstrate that out of 301 metabolites, the suppression of specific PC levels are most associated with fatty liver disease). Data were obtained using time-of-flight mass spectrometry. Changes in TG-rich lipoprotein (B) PC and (C) TG in response to RP choline ion feeding (study described by Zenobi et al., 2018). Data derived using fast-protein liquid chromatography and time-of-flight mass spectrometry. Although the TG-rich lipoprotein fraction is believed to be primarily VLDL, the fraction may contain intestinal-derived chylomicrons.

If choline does not activate PEMT, can other methyl donors like methionine or betaine serve this function? Zhou and coworkers (2018) compared choline versus methionine supplementation in primary bovine liver cells. They observed marked elevations in MAT1A and PEMT protein abundance in liver cells treated with methionine. Additionally, Osorio and colleagues (2014a) demonstrated the ability of RP methionine to increase hepatic MAT1A mRNA expression in transition cows. Collectively, the current body of literature supports the hypothesis that choline and methionine uniquely activate the CDP-choline and PEMT pathways, respectively. Therefore, the combined supplementation of RP choline and methionine may be warranted to activate both pathways of PC synthesis and thus optimize liver TG secretion. Likewise, RP choline and betaine co-supplementation has the potential to elicit similar outcomes. Unfortunately, studies investigating co-supplementation of these nutrients are limited. We have demonstrated that RP methionine, choline, and betaine co-supplementation slows the postpartum progression of hepatic lipid deposition in Holstein cows (Zang et al., 2018). Also, the combined dietary supplementation of RP choline and methionine has been shown to increase plasma VLDL and Apo B<sub>100</sub> concentrations in postpartum cows, relative to choline or methionine alone (Sun et al., 2016). However, gains in performance (e.g., milk yield) were not elevated with co-supplementation (Zhou et al., 2016). Potentially independent of choline status, RP methionine supplementation has other potential benefits to consider including improvements in milk protein synthesis, elevated carnitine status to support FA  $\beta$ -oxidation, greater plasma oxygen radical absorbance capacity, and reduced inflammation (Osorio et al., 2014b; Zhou et al., 2016).

#### *Fatty acid strategies that may modulate the hepatic phosphatidylcholine profile*

Because the CDP-choline and PEMT pathways generate PC with a unique FA composition, then it is conceivable that the hepatic FA profile influences the efficacy of choline and methionine to activate these pathways. It can be hypothesized that FA substrate supply for the CDP-choline pathway is met by the lipolytic release of palmitic and oleic acids from adipose tissue. Moreover, we can hypothesize that the delivery of polyunsaturated FA such as omega-3 DHA would selectively provoke PEMT activation and transmethylation. Such action would be advantageous for the transition cow because hepatic concentrations of DHA-containing phospholipids are lowest at calving (Douglas et al., 2007). To explore the ability of DHA to modulate PC production, five multiparous lactating Holstein cows were continuously abomasally infused for 6 d with emulsion preps containing (i) palmitic acid (PA; 98% C16:0; BergaFat F-100 HP; Berg + Schmidt GmbH & Co.), (ii) PA + choline chloride (PA+C; 50 g/d choline chloride; Balchem Corporation), (iii) PA + L-serine (PA+S; 170 g/d L-serine; 1X predicted duodenal flow; Hard Eight Nutrition), (iv) behenic acid (BA; 92% C22:0; Berg + Schmidt GmbH & Co.), or (v) an algal oil rich in docosahexaenoic acid (44% C22:6 n-3; algae-sourced life'sDHA; DSM Nutritional Products, Inc.). Although each cow was infused 301 g of total FA each day (12.54 g/h), infusions were balanced for the amount of C16:0 and glycerol within the omega-3 oil (40 and 19 g, respectively). Each emulsion prep contained whey protein, polysorbate 80, ethoxyquin, and water. When compared with cows infused PA, the majority of PC (61 PC out of 114 PC detected) in liver were more abundant in DHA-infused cows. Specifically, the amounts of hepatic PC containing polyunsaturated FA including DHA (52 PC out of 61 PC; e.g., PC-14:0/22:6) were greater with the infusion of DHA, relative to PA (Figure 3A). Also, the hepatic amounts of PC containing FA not found or found in negligible amounts in the emulsion were highest in cows infused with DHA (e.g., PC-14:0/18:3; Figure 3B). Although not observed with DHA infusion, a modest number of PC with a high degree of saturation were

elevated with PA+C infusion, relative to PA (18 PC out of 114 PC detected; not shown). The effects of PA+S or BA infusion on hepatic PC were also modest, relative to PA (not shown). Collectively, our data support the previously stated hypothesis that choline principally activates the CDP-choline pathway to generate saturated PC. Whereas our data suggest that DHA activated PEMT. Deserving further investigation, RP choline and omega-3 FA co-supplementation in transition cows may be a means to activate both pathways of PC synthesis and thus maximize VLDL export. Additionally, dietary omega-3 FA supplementation may be a means to enhance methionine's ability to promote transmethylation. Such possibilities will need to ensure the adequate protection of DHA to avoid undesirable effects on rumen biohydrogenation and milk fat production. The dietary supply of the vitamin E antioxidant will need to be considered with this strategy.



**Figure 3.** Effect of abomasal infusion of palmitic acid (PA; 16:0), PA and choline chloride (PA+C), PA and L-serine (PA+S), behenic acid (BA; 22:0), or a mixed omega-3 oil containing docosahexaenoic (DHA) on hepatic (A) phosphatidylcholine (PC)-14:0/22:6 and (B) -14:0/18:3 levels in late lactation Holstein dairy cows. Generalized log-transformed intensities (Y-axis) derived from time-of-flight mass spectrometry. Samples reflect liver biopsied at the end of d 6 of each infusion. See text for treatment description details.

In humans, steatohepatitis is an inflammatory subtype of non-alcoholic FLD which is characterized by PC depletion. Interestingly, the mechanisms involve the activation of an enzyme called acid sphingomyelinase which transforms sphingomyelin into ceramide. The activation of acid sphingomyelinase is potentially relevant because it (1) downregulates *S*-adenosylmethionine synthesis and PEMT activation via the inhibition of MAT1A and (2) triggers the deactivation of the CDP-choline pathway via ceramide-dependent mechanisms (Marí et al., 2004; Garcia-Ruiz et al., 2015). This is potentially alarming for the transition cow because palmitic acid is a toll-like receptor 4 ligand that increases inflammatory tumor necrosis factor- $\alpha$  production, which happens to be a potent inducer of acid sphingomyelinase. Therefore, excess lipolytic- or dietary-derived palmitic acid may inadvertently challenge hepatic PC production. Because lipolysis and dietary

palm fat feeding promotes ceramide accrual in cows (Rico et al., 2016; Davis et al., 2017b), the ability of palmitic acid to thwart PC production deserves investigation.

## Summary

The transition cow is prone to excessive body fat mobilization and FLD which may be detrimental to health, milk production, fertility, and longevity. We must understand that the biological effect of “fat feeding” likely depends on the type of FA fed. Increasing the dietary proportion of saturated fat may accelerate postpartum body weight loss and inadvertently challenge hepatic health. The periparturient inclusion of unsaturated FA such as omega-3 FA, preferably in a protected form, has potential to control lipolysis to support health and production. Moreover, dietary methyl donors including choline, methionine, and betaine may be a means to enhance hepatic TG disposal and thus minimize liver injury in the form of inflammation and oxidative stress. Emerging evidence also suggests that omega-3 FA supplementation may be an approach to optimize methyl donor efficacy and hepatic metabolic health. Methyl donor and FA nutrition should be considered in parallel to help the dairy cow achieve superior performance.

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## **Improving Immunity in Dairy Cows During Times of Stress Through Nutrition**

### **Utiliser la nutrition pour améliorer l'immunité chez les vaches laitières en période de stress**

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#### **Abstract**

There are many factors that increase the risk for infectious diseases during the transition period. If a cow is able to adapt to all the physiological, nutritional, environmental, and social changes that occur during the transition period she will be more productive during that lactation and more likely to reach the next one. Therefore, we must take a systematic approach to understand what influences immunity, so that we can increase the odds that cows will successfully navigate this important period, ultimately increasing production efficiency of the dair .

First, we need to understand what aspects of the immune system are compromised that lead to the increased susceptibility to infectious diseases. There are many components and layers that make up a cow's immune system, including: physical barriers, antimicrobial secretions, and many cellular responses. The immune system is complicated and the competency of the system is a function of many interactions. Infections with environmental microorganisms are common around calving, primarily resulting from holes in the physical barriers of those tissues that are associated with milking and calving. Disease likely will not ensue if other aspects of the immune system can control the growth and ultimately eliminate the microorganism from that tissue. Unfortunately, other immune defenses are compromised or what I consider dysfunctional, which increases the risk the infection will develop into disease.

Cows are exposed to many potential stressors around calving, some physiological (i.e. associated with metabolic demands of lactation) and others social. If cows are overwhelmed by the number and/or severity of the stressor(s), various leukocyte responses may become compromised. Stressed animals were shown to have altered leukocyte responses. Cows stress about change and situations that create competition among cows can also create winners and losers, which can increase the risk for disease among some animals. Parturition and subsequent lactation are abrupt; therefore, management strategies need to try and make that change less dramatic and limit additional stressors that may interfere with the ability of the cow to adapt.

Additionally, metabolic demands of leukocytes may not be prioritized or sufficiently met around calving. Neutrophil functions of sub-clinically hypocalcemic cows were reduced during early lactation. There also is evidence that elevated NEFA and BHBA concentrations have a negative impact on immunity. Therefore, management strategies that can improve both calcium and energy homeostasis will improve the success rate of cows during the transition period.

## Résumé

Plusieurs facteurs peuvent augmenter le risque de maladies infectieuses durant la période de transition. Si une vache peut s'adapter à tous les changements physiologiques, nutritionnels, environnementaux et sociaux qui surviennent durant la période de transition, elle sera plus productive durant cette lactation et plus susceptible d'atteindre la suivante. Par conséquent, nous devons adopter une approche systématique pour comprendre ce qui influence l'immunité, afin de pouvoir augmenter les chances que les vaches traversent avec succès cette période importante, augmentant du même coup l'efficacité de la production laitière

D'abord, nous devons comprendre quels aspects du système immunitaire sont compromis et mènent à une plus grande susceptibilité aux maladies infectieuses. Plusieurs composantes et couches forment le système immunitaire d'une vache, incluant : barrières physiques; sécrétions antimicrobiennes; et plusieurs réponses cellulaires. Le système immunitaire est complexe et sa compétence dépend d'une multitude d'interactions. Les infections aux micro-organismes environnementaux sont courantes dans le temps du vêlage, causées principalement par des trous dans les barrières physiques des tissus associés à la lactation et au vêlage. La maladie ne s'ensuivra probablement pas si d'autres aspects du système immunitaire peuvent contrôler la croissance et ultimement éliminer les micro-organismes de ce tissu. Malheureusement, d'autres défenses immunitaires sont compromises ou, selon moi, dysfonctionnelles, ce qui accroît le risque que l'infection se développe en maladie.

Les vaches sont exposées à plusieurs stressseurs potentiels au temps du vêlage, certains physiologiques (c.-à-d. associés aux demandes métaboliques de la lactation) et d'autres sociaux. Si les vaches sont accablées par le nombre et/ou la gravité des stressseurs, diverses réactions des leucocytes peuvent devenir compromises. Les animaux stressés ont montré des réactions leucocytaires altérées. Le changement est cause de stress pour les vaches et les situations qui créent de la compétition entre elles peuvent aussi créer des gagnantes et des perdantes, ce qui peut augmenter le risque de maladie chez certains sujets. Comme la mise bas et la lactation subséquente sont abruptes, on doit appliquer des stratégies de gestion pour essayer de rendre ce changement moins dramatique et pour limiter les stressseurs additionnels qui peuvent nuire à la capacité de la vache de s'adapter.

En outre, les demandes métaboliques de leucocytes peuvent ne pas être priorisées ou suffisamment satisfaites au temps du vêlage. Les fonctions des neutrophiles chez les vaches subcliniquement hypocalémiques se sont réduites au début de la période de lactation. On a aussi observé que des concentrations élevées d'AGNE et de BHBA ont un impact négatif sur l'immunité. Par conséquent, les stratégies de gestion qui peuvent améliorer le calcium et l'homéostasie énergétique augmenteront le taux de succès des vaches durant leur période de transition.

## Introduction

The immune system is made up of many components including: various physical barriers, antimicrobial secretions, and cellular responses. A breakdown in any aspect of the immune system in the presence of a pathogen may increase the likelihood of infectious disease. It is well established that dairy cattle are highly susceptible to infectious diseases affecting many tissues during the transition period, and stress is often considered in the etiology. The word stress is commonly used, but the term itself can take on many different meanings and therefore the use is often vague or an over-generalization. As we alluded to in the previous sentence, stress is commonly referred to in the etiology of infectious disease in cows; therefore, stress is considered negative in the context of dairy cattle health. In contrast, stress is a natural, physiological response that is important in promoting a response to a threat or adaptation to a change (e.g. the transition period). Therefore, the paradox is that stress is likely both crucial for the adaptation to lactation to occur, but potentially damaging to the transition dairy cow.

This presentation will consider what are potential sources of stress for transition cows and will further describe how these potential stressor(s) influence immune defenses and risk for infectious diseases. To address this topic we will first describe the basic framework of the immune system and will briefly describe how the immune system of many transition cows differs from that of either a non-lactating or mid to late lactating cows. Then, we will attempt to define stress, describe possible stressors a transition cow may encounter, and investigate the potential role that various stressors alone or in combination influence immune defenses and risk for infectious diseases.

## Periparturient Cow Immune System

The principle role of the immune system is to recognize self from non-self, and in doing so protect the cow's organs against infectious, non-self microorganisms such as bacteria, virus, parasites, and fungi. The immune system is exactly that, a system, which is made up of many components. Understanding how any single measurement of the immune system influences the risk for disease is complicated because a break down in any component of the immune system may either increase the risk for disease or have no affect at all under the specific circumstances. The immune system has various layers ranging from the physical barriers to very specialized leukocyte functions. The physical barriers are often compromised (e.g. an open teat end and microbial contamination of the uterus), which contributes to the susceptibility to mastitis and uterine diseases in early lactation. However, if other components of immune system are functioning properly a mild infection is eliminated without any clinical signs of disease. An example of this was observed when Shuster et al. (1996) challenged cows intra-mammary in either early lactation, 6 to 10 DIM, or in mid lactation with the same strain and dose of *E. coli* and observed that the cows in early lactation had greater replication of the *E. coli* and developed more severe mastitis. Therefore, it is important to understand what other aspects of the immune system are compromised during the transition period that increases the risk for infectious disease.

Ballou (2012) described the immune system of many transition cows as dysfunctional. The compromised physical barriers when coupled with suppressed ability to control the growth of microorganisms in tissues increase the risk of clinical and sub-clinical diseases. Suppressed lymphocyte and neutrophil functions during the transition period are well documented in many

studies from different lab groups (Guidry et al., 1976; Mallard et al., 1998; Burvenich et al., 2003). Additionally, other aspects of the innate immune system beyond lymphocyte and neutrophil functions appear to be suppressed during the transition period. Shuster et al. (1996) reported a reduced ability to control the growth of the *E. coli* in the mammary gland when the cows were challenged in early lactation, and this occurred before the recruitment of neutrophils. Additionally, Ballou et al. (2009) reported that whole blood bactericidal capacities against both an environmental *E. coli* and a pathogenic *Salmonella typhimurium* were reduced the day after calving and returned to prepartum levels by 21 DIM. In contrast, one aspect of the immune system that does not appear to be suppressed during the transition period is the inflammatory response. Lehtolainen et al. (2003) reported that cows in early lactation had greater local and systemic signs of inflammation after they were challenged with a large dose, 100 µg, of lipopolysaccharide intra-mammary. In agreement, Sordillo et al. (1995) reported greater *ex vivo* secretion of tumor necrosis factor- $\alpha$  when stimulated with lipopolysaccharide. These studies are important pieces of evidence for an increased propensity to produce inflammation in early lactation because both of these models did not use live microorganism challenges, but instead challenged with a fixed dose of an agonist that activates the inflammatory responses. Therefore, the immune system of a transition cow is dysfunctional because some responses are suppressed, whereas the inflammatory response appears elevated. This immunological phenotype is in contrast to a generalized immunosuppression (Ballou, 2012). This distinction is important when evaluating the role that transition period stress plays in the increased risk for disease.

## **Role of Stress in Periparturient Disease**

Stress has for a long time been implicated in the etiology of many infectious diseases both in humans and dairy cattle, but how much does stress during the transition period actually play in the elevated risk for disease? Is the importance of stress in transition cows exaggerated? In order to establish a causal link between stress and impaired health during the transition period, there are a series of assumptions or steps that must be met, including: (1) the cow is stressed, (2) the classical stress response is elicited, (3) the duration or magnitude is sufficient to alter various immune functions, and (4) that increases the risk of getting an infectious disease, either clinical or sub-clinical. Further, alternative pathways from the first to the last step need to be considered. We will use this framework to investigate the role that stress may play in increasing the risk for infectious disease during the periparturient period.

Therefore, the first step is to understand what stresses a cow. In order to address this question we need to define stress, which is not as straightforward as one might expect. Stress is referred to in many contexts and the meaning is often subjective. Hans Selye was the first to coin the term stress in 1936 and he defined it as, “the non-specific response of the body to any demand for change”. Selye performed experiments in laboratory animals and observed consistent pathological changes in animals, lymphoid atrophy, stomach ulcers, and enlargement of the adrenal glands, in response to various psychological and physiological challenges. We will accept his original definition to evaluate what are potential stressors that a cow is exposed to during the transition period. The commonly used term, transition period, already appears to validate the first assumption that cows are exposed to stress during this time. Any transition involves change, so the next question is, what changes are taking place during this period?

Cows are not that different from humans in what causes stress. Have you ever wondered why cows are creatures of habit? It is the same reason that humans are creatures of habit or that humans are most comfortable when they are in a routine. It basically boils down to control. Change or uncertainty causes a loss of control, whether you are a human or a dairy cow. Common laboratory models of stress involve taking control away from the subject. An example would be put a loud alarm in a room housing animals that goes off randomly throughout the day. The alarm must be set to go off randomly so the subjects cannot adapt to the alarm. By setting the alarm to go off at random, the animals have no sense of control. In contrast, if the alarm goes off at a regular interval, the subjects regain control of the situation and therefore will adapt. Other common laboratory stress models include physical restraint, social re-organization, or inability to move away from a painful stimulus. If we apply the principle that a significant change can cause a loss of control and the stress response is the physiological response to help the animal regain control, then we must understand what changes are potentially stressful to transition dairy cows. Generally, the changes that occur during the transition period are primarily either psychological or physiological.

Let's first consider the psychological or social stressors that a dairy cow may encounter during the transition period. Many potential pen moves occur throughout the transition period. Cook and Nordlund (2004) described these pen moves and how after every pen change there is the potential for social re-organizing that persists for 3 to 7 days. von Keyserlingk et al. (2008) reported increased competition at the feed bunk, decreased lying bouts, and reduced allogrooming events the day after a single lactating cow was introduced into a stable population of 11 lactating cows. The same group also observed a 9% decrease in DMI on the day that a dry cow was moved to a new pen when compared to baseline values (Schirmann et al., 2011). Further, they reported that the new cows displaced other cows at the feed bunk twice as much as they did before they were moved. The displacement behaviors are noteworthy because they indicate competition or aggressive/submissive actions. Dominant lactating cows when moved to a new pen did not change their behavior or drop milk production; however, intermediate and subordinate cows produced 3.8 and 5.5% less milk, respectively during the 2nd week after pen moves (Hasegawa et al., 1997). In contrast, Chebel et al. (2016) reported that highly dominant cows with multiple interactions at the feed bunk throughout the day were more likely to have uterine disease and be culled from the herd.

Most dairy cows are raised in confinement and the temptation to maximize facility space can result in overstocking. We'll define overstocking as the number of cows per pen exceeds available resources (i.e. access to feed and/or a comfortable place to rest), which creates unnecessary competition. When management creates competition among cows, there are winners, but there are also losers. This will likely increase the risk that the subordinate or overly dominant cows will be stressed and/or have other negative health and productive outcomes.

In addition to the psychological stressors, there are many physical changes taking place in the cow during the transition period that can elicit a stress response. Nutrient and energy demands increase during lactation. Additionally, energetic demands approximately double during lactation and most early lactation cows will be in some degree of negative energy balance. Antioxidants are also used at a greater rate and can cause depletion of antioxidant stores (Weiss et al., 1997). Similarly, increased calcium output in colostrum and milk can cause a rapid drop in ionized calcium in blood, until allosteric mechanisms activate osteoclasts to mobilize calcium from bone. There is evidence that these metabolic changes during the transition period have both direct and indirect effects on leukocyte responses (Lacetera et al., 2004; Moyes et al., 2009; Zarrin et al., 2014).

It is evident from the previous discussions that dairy cows during the transition period are exposed to many changes, both psychologically and physically that are potentially stressful. However, just because something is potentially stressful or even that behaviors of cows change does not necessarily mean that cows are stressed and further that immune function is altered and disease risk increased. Silva et al. (2016) reported an increased frequency of adverse behaviors when stocking density increased; however, they did not observe any differences in leukocyte function or incidence of periparturient disease. The author's suggested that although increasing stocking density may have been a mild stressor in this population, the overall good management of this herd did not make this stressor overwhelm the ability of the cows to cope with additional stressors associated with the periparturient period.

There is good evidence that cows are exposed to many changes that are potential stressors during the transition period. Additionally, activation of the stress response occurs around parturition; however, the impacts on immunity are not completely clear. Increased risk for disease persists past the period of elevated plasma cortisol in cows as well as leukocytes may be less responsive to glucocorticoids around calving because the glucocorticoid receptor is down regulated in those cells. Therefore, future research will need to delineate between stress and alternative routes that result in increased disease risk.

### **Alternative Routes of Increased Periparturient Disease Susceptibility**

Immune responses can be metabolically expensive and requirements may be limiting for optimal function during the periparturient period. Evidence from Martinez et al. (2013) indicated that cows classified as subclinical hypocalcemic (total serum calcium less than 8.59 mg/dL) had less reactive neutrophil oxidative burst when compared to normocalcemic cows. Further, they reported increased incidences of metritis and extended days to confirmed pregnancy in the subclinical hypocalcemic cows. These data indicate that even among subclinical hypocalcemic cows, leukocyte function may be impaired and increase the risk for periparturient disease. Interestingly, in our lab if we collect blood using an anticoagulant that chelates calcium, we are unable to activate neutrophils in whole blood.

There is also evidence that elevated NEFA and BHBA concentrations have a negative impact on immunity. Lacetera et al. (2004) reported that inclusion of NEFA in cell culture media as low as 0.25 mM reduced IgM secretion. Further, they reported attenuated mitogen induced interferon- $\gamma$  secretion when the cell culture media included as low as 0.125 mM NEFA. Moyes et al. (2009) induced negative energy into post-peak cows by partially restricting feed intake. The feed restriction increased plasma NEFA and BHBA to levels common among periparturient cows. The cows were challenged intramammary with an environmental *Streptococcus uberis* and pathophysiological response determined. The cows in negative energy balance had reduced neutrophil phagocytosis immediately before the mastitis challenge and had elevated acute phase protein concentrations after the challenge. This indicated that the cows in NEB had greater inflammatory response to the mastitis challenge. More recently, Zarrin et al. (2014) infused cows with BHBA to induce hyperketonemia and infused control cows with normal saline. All cows were challenged intramammary with lipopolysaccharide in order to evaluate the intensity of the acute phase response. The hyperketonemia cows had elevated acute phase protein secretion and reduced the influx of somatic cells into the mammary gland after the lipopolysaccharide challenge. The

reduced influx of somatic cells into the mammary gland after an infection would increase the risk for development of mastitis and as well as the severity of mastitis.

Lastly, increased metabolic activity and leukocyte derived oxidant production during early lactation can accelerate the use of antioxidants, and if it exceeds the supply of antioxidants can result in some degree of oxidative stress. The implications of this oxidative stress can have negative impacts on the immune responses of cows and ultimately disease resistance.

## **Implications**

The immune dysfunction of periparturient dairy cows is complex, and appears to involve many layers of the immune system. Increased exposure of microorganisms occurs from both calving and milking; however, a competent immune system should be able to eliminate most infections without any clinical disease. Many psychological and physiological stressors appear to be involved in increasing the risk for infectious disease; however, it appears to be somewhat of a cumulative effect. Some stress is unavoidable, but the goal should be to limit additional stressors that may ultimately impair the ability of a cow to cope with the change from non-lactating to lactating. In addition to stress, changes in nutrient supply or use may impair leukocyte function and ultimately increase the risk for infectious disease. Therefore, there is not a single source of immune dysfunction during the periparturient period. Management must look at each production system separately and take a systematic approach to improving transition success.

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# Conference Colloque



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## **The Impact of ‘-omics’ Technologies on Nutrition**

### **L’impact des technologies en « -omique » sur la nutrition**

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#### **Abstract**

We currently live in an era of Big-Data, as consumers in the marketplace, as biomedical patients, and as animal scientists. Omics technologies including genomics, transcriptomics, proteomics, metabolomics, microbiomes, and metagenomics are increasingly becoming integral to the understanding and implementation of nutritional strategies in animal agriculture. These technologies are being implemented to expand the understanding of individual nutrients (molecules) to the complex response to the addition of exogenous microbes (probiotic cocktails) to animal diets potentially eliciting effects far beyond the gastrointestinal tract. In the following review, contributing research, developed at the interface of nutrition and the Omics technologies, will be discussed using examples and practical implications relevant to animal production systems.

#### **Résumé**

Nous vivons présentement à l’ère des mégadonnées, en tant que consommateurs sur le marché, patients biomédicaux et scientifiques spécialistes des animaux. Les technologies omiques incluent la génomique, la transcriptomique, la protéomique, la métabolomique, les microbiomes et la métagénomique, qui deviennent de plus en plus intégraux pour comprendre et implanter des stratégies nutritionnelles dans l’élevage des animaux. On implante ces technologies afin de mieux connaître les nutriments individuels (molécules) dans leur réaction complexe à l’ajout de microbes exogènes (cocktails probiotiques) aux régimes des animaux, élicitant potentiellement les effets bien au-delà du tube digestif. Dans l’examen qui suit, la recherche contributive, développée à l’interface de la nutrition et des technologies omiques, fera l’objet d’une discussion, accompagnée d’exemples et d’implications pratiques pertinentes aux systèmes de production animale.

# 2019 ANCC Kees de Lange Lectureship in Animal Nutrition

A truly outstanding scientist, colleague and mentor, Prof. Cornelis (Kees) F.M. de Lange was always thinking about the future and how to better the industry through research. His legacy of excellence, originality and industry support is one that won't be forgotten by his colleagues, students and friends.

Dr. de Lange joined the faculty of the Department of Animal Biosciences (formerly Animal and Poultry Science) at the University of Guelph in 1994 as a professor of Swine Nutrition. He had a highly productive and well respected career with over 150 peer-reviewed publications, 130 presentations as well as numerous internal, national and international awards. Dr. de Lange was also a tremendous educator to undergraduate teaching and supervised more than 50 graduate students and postdoctoral fellows.



Prof. Kees de Lange

Following his passing on August 1, 2016, and in honour of Dr. de Lange's accomplishments, an endowed graduate scholarship in swine nutrition was created by friends, family and colleagues. As a testament to the impact Kees had on those around him, donors rapidly came forward and raised more than \$150,000.

The 2019 ANCC Kees de Lange Lectureship in Animal Nutrition is another fitting tribute to the significant contributions made by Dr. de Lange to research and the greater food animal agriculture community.



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## Microbial Sensing: Implications for Gut Health in the Neonate

### Détection des microbes : répercussions sur la santé intestinale des nouveau-nés

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#### Abstract

There is considerable agreement that the gastrointestinal microbiota contribute to the performance and health of the neonate. This relationship establishes the ability of the host animal to “sense” microorganisms, affecting changes in physiological response. Our challenge, however, continues to be to understand which organisms, among the many present, should be promoted to improve gut functions including nutrient assimilation and pathogen protection. Establishing the mechanisms used by the host to sense microbiota is one approach to meeting this challenge. Diet independent microbial products are molecules unique to the microbial community biomass (endotoxin, peptidoglycan, flagellin) and sensed by host Pattern Recognition Receptors stimulating inflammation. As these molecules are common among all members of the microbial community. Thus responding differently to organisms that may be a friend or a foe, depends on the microenvironment in which the molecule is detected. Diet dependent microbial products arise as products of fermentation of dietary components (e.g. protein, fibre) and include short chain fatty acids, ammonia, phenols, H<sub>2</sub>S, amines and many other compounds. A plethora of sensing mechanisms exist, consistent with the diversity of molecules. These include enzymatic metabolism as well as membrane receptors that have evolved to respond to microbial products (e.g. short chain fatty acid receptors), or receptors that respond to microbial products because the natural ligand is mimicked by a microbial ligand (histamine). We will review host mechanisms used to sense the intestinal microbiota and attempt to establish practical considerations for neonatal gut health based on current understanding.

#### Résumé

On s'entend généralement pour dire que le microbiote gastro-intestinal contribue au rendement et à la santé du nouveau-né. Cette relation détermine l'aptitude de l'animal hôte à « sentir » la présence des microorganismes, conditionnant les changements de la réponse physiologique. Notre défi demeure cependant de comprendre quels organismes, parmi les nombreux qui sont présents, devraient être favorisés pour améliorer les fonctions intestinales, y compris l'assimilation des nutriments et la protection contre les agents pathogènes. Déterminer les mécanismes utilisés par l'hôte pour détecter le microbiote est une façon de relever ce défi. Les produits microbiens indépendants du régime alimentaire sont des molécules uniques à la biomasse de la communauté microbienne (endotoxine, peptidoglycane, flagelline) et sont détectées par les récepteurs hôtes de reconnaissance de formes inflammatoires. Ces molécules sont communes parmi les membres de la

communauté microbienne. Ainsi, répondre différemment à des organismes qui peuvent être amis ou ennemis dépend du microenvironnement dans lequel la molécule est détectée. Les produits microbiens dépendants de l'alimentation sont des produits issus de la fermentation de composants alimentaires (protéines et fibres, par exemple) et comprennent les acides gras à chaîne courte, l'ammoniac, les phénols, le H<sub>2</sub>S, les amines et de nombreux autres composés. Il existe une pléthore de mécanismes de détection correspondant à la diversité des molécules. Il s'agit notamment du métabolisme enzymatique ainsi que des récepteurs membranaires qui ont évolué pour répondre aux produits microbiens (p. ex., les récepteurs des acides gras à chaîne courte) ou des récepteurs qui réagissent aux produits microbiens parce que le ligand naturel est imité par un ligand microbien (histamine). Nous passerons en revue les mécanismes hôtes utilisés pour détecter le microbiote intestinal et tenterai d'établir des considérations pratiques applicables à la santé intestinale du nouveau-né en me fondant sur les connaissances actuelles.

## Introduction

The gastrointestinal tract (GIT) is home to a complex microbial ecosystem that represents a vast metabolic capacity that responds to changes in environment, diet composition, and genetics and that significantly influences the host animal. A single layer of epithelial cells separates the luminal microbiota and their metabolites from the underlying tissues of the body and plays a key role in sensing and responding to changes in the luminal environment (Wells et al., 2010; 2011). Just beneath this epithelial layer is a layer of organized immune cells that stand ready to clear microbial “trespassers” that have penetrated the epithelial barrier. Thus, the intestinal mucosal epithelium is often described as a “barrier” and discussions of gut health often refer to “gut barrier function”. Of course, nutritionists are well aware that the epithelium must have selective permeability, serving as a barrier to trespassing microbes and their toxins while also facilitating efficient digestion and absorption of nutrients for growth and maintenance. Thus, the two primary functions of the gastrointestinal tract, nutrient assimilation, and microbial barrier, are interdependent. Furthermore, host mechanisms to sense changes in the gut microbial ecosystem result in changes to both primary functions of the GIT and should be considered when assessing gut health.

Brestoff and Artis, (2013) have suggested that the cross-talk between the intestinal epithelium and the GIT microbiota includes epithelial and immune cell sensing of both the microorganisms themselves and the products of microbial metabolism or fermentation. Furthermore, these authors suggest that host sensing microbial components should be considered as diet-independent as these components are not markedly affected by changes in diet composition and demonstrate greater dependence on environment. Host sensing of microbial components is achieved through the detection of molecular patterns unique to microbiota (termed microbiota-associated molecular patterns or MAMPS) by families of host pattern recognition receptors (PRR) present on epithelial cells and immune cells (Sellge and Kufer, 2015). Alternatively, microbial metabolites are generated primarily through fermentation of dietary components and therefore should be considered as diet-dependent (Brestoff and Artis, 2013). Host sensing of diet-dependent changes in microbial metabolites occurs by a large variety of mechanisms that are difficult to categorize but include receptor-mediated mechanisms (e.g. short chain fatty acid receptors), enzymatic metabolism and cell toxicity (e.g oxidative stress, cell membrane damage). Host sensing mechanism for both diet dependent and diet independent microbial products contribute to changes in gut health. This paper

will review host sensing mechanisms responding to changes in the GIT microbiota to help inform nutrition and management strategies, particularly in the neonate.

### **Diet independent microbial sensing**

Sensing of components of microorganisms in the GIT is mediated by PRR binding of MAMPS which are present on both commensal bacteria and pathogens as well as viruses, yeasts, and other microorganisms. Commensal bacteria are known to stimulate recruitment of immune cells, regulate immune functions such as mucus and antimicrobial peptide secretion (Macpherson and Harris, 2004; Hooper and Macpherson, 2010). For example, *Bacteroides fragilis* has been reported to promote a balance in T helper cells, subsequently promoting T-helper 1 development associated with cell-mediated immunity (Mazmanian et al., 2005; Round and Mazmanian, 2010). There is also evidence that bacteria in Clostridia Cluster IV and XIVa promote the development of immunosuppressive T regulatory cells (Zeng and Chi, 2015). Furthermore, *Bacteroides thetaiotamicron* has been reported to induce Reg3 $\gamma$ , an antimicrobial peptide which acts primarily against Gram-positive bacteria (Cash et al., 2006). Indeed, the dramatic contribution of a conventional commensal bacterial to gastrointestinal tract development has been confirmed using gnotobiotic pigs (Shirkey et al. 2006). Furthermore, this work also established that different commensal bacteria evoke different developmental responses (Willing and Van Kessel, 2009).

What is clear is that while we generally consider pathogens that are capable of penetrating host barriers as initiators of inflammation and immune response, non-pathogenic commensal bacterial are also detected by the host and modulate immune response. Obviously, mechanisms must permit the differentiation of commensal (friends) and pathogenic (foe) bacterial species (Van den Abbeele et al., 2011) to avoid unnecessary activation of otherwise damaging inflammatory responses. Given that PRR is the primary mechanisms of sensing of microorganisms by the host, and that MAMPs are present on both pathogens and non-pathogens, differentiation of friend and foe require additional mechanisms. Generally, this differentiation is ascribed to the context in which PRR is activated by MAMP (Artis, 2008). In this context, microorganisms detected on the host side of the epithelial barrier might be recognized as a foe, leading to an active inflammatory response whereas organisms first detected on the luminal side of the barrier, induce a more moderate and regulatory immune response. The concept that commensal bacteria are ignored by the host immune system is further challenged by the observation that secretory IgA, immunoglobulins secreted into the gut lumen and traditionally associated with binding to pathogens and preventing their attachment, also bind to a subset of commensal bacteria (Pabst et al. 2016). The implications of these observations remain to be established but it is interesting that IgA binding may enhance bacterial uptake across specialized M cells present in the intestinal epithelium for recognition by the acquired arm of the immune system.

The signaling receptors (PRRs) on host cells include the Toll-like receptor family (TLRs), the nucleotide oligomerization receptor (NOD) family, the NOD-like receptor family (NLRs) and RIG-1 like receptor family. A review by Sellge and Kufer (2015) categorized PRRs into humoral receptors (found in serum), membrane-bound receptors (involved in responding to extracellular MAMPS) and endosomal and cytosolic receptors (react to intracellular MAMPS). These receptors are located on the intestinal epithelial cells (IECs) and immune cells such as the dendritic cell of the innate immune system. Recognition of microbes by these cells through their PRRs initiates an

inflammatory response and modulates the activation of immune cells such as T cells, to elicit an appropriate acquired immune responses (Didierlaurent et al., 2006; Zhu et al., 2012; Thaïss et al., 2016) reflecting the context of activation. For example, activation of intracellular PRR indicate a clear breach of the epithelial barrier and would, therefore, contribute to inflammation and activation of an acquired immune response.

In summary, the immune system directly recognizes microbes that transverse the gut barrier by activating PRR resulting in enhanced immediate innate defense (e.g. mucus secretion, increased epithelial cell replacement, antimicrobial peptide secretion) but also generating antibody and T cell responses through acquired immunity that includes memory responses in case of a subsequent infection. Both innate and acquired immune mechanisms also respond to microbes which do not normally traverse the gut barrier, through activation of PRR. Thus both pathogens and commensal organisms are “sensed” by the host and modulate immunity. These observations suggest that approaches to manipulate the members of the gut commensal microbial community under healthy conditions could be an approach to modulate intestinal immune function to provide benefit in case of pathogen exposure.

### **Diet-dependent microbial sensing**

Diet is well established as one of the major external modulators of gut microbiota, whereas the gut microbiota possesses an array of enzymes that contribute to digestion associated with vitamin synthesis, lipid metabolism and production of numerous metabolites. Non-ruminants, including pigs, cannot digest most of the complex carbohydrates found in their diet but microbes inhabiting the gut possess catabolic enzymes that can degrade and ferment a wide array of these carbohydrates (Jacobs et al., 2009). Dietary protein, digestive enzymes, mucins and bile acids that escape ileal digestion can also be fermented or bio-transformed in the hindgut (Jacobs et al., 2009; Dai et al., 2011) or transformed by small intestinal microbiota during transit. Generally, carbohydrate fermentation is considered beneficial to the host, whereas protein fermentation is associated with potentially detrimental effects based on the metabolites generated, microflora microbiota involved, and their impact on gut health (Jacobs et al., 2009). Furthermore, many of these diet dependent microbial-derived products have been reported to serve as signaling molecules that influence metabolic and immune responses both systemically and, in the gut, (Rooks and Garrett, 2016). In what follows, we review the sensing mechanisms for a number of diet-dependent microbial products and their implications for gut health.

The gut microbes can ferment complex polysaccharides (i.e., oligosaccharides, resistant starch, and some non-starch polysaccharides referred to as dietary fiber; DF) that escape digestion. The non-digestible carbohydrates, upon reaching the hindgut, favor the growth and metabolism of fibrolytic bacteria such as *Bifidobacterium* spp., *Lactobacillus* spp., and *Roseburia* spp., which are considered to be beneficial for gut health due to their health-promoting properties (Haenen et al., 2013a; Jha and Berrocoso, 2015; Knudsen et al., 2016). The main metabolites produced from microbial fermentation of carbohydrates in the gut consists of short chain fatty acids (SCFA) and variable amounts of H<sub>2</sub>, carbon dioxide, and methane (Jacobs et al., 2009). The SCFA are absorbed into epithelial cells by passive diffusion across the cell membrane and through transporter-mediated pathways (Haenen et al., 2013b; Nielsen et al., 2015). Absorbed SCFA is metabolized locally for energy or deposited in the port vein for transport to the liver. The SCFA are also sensed by specific

membrane-bound receptors known as G protein-coupled receptors (GPR) 41, 43 and 109A (Besten et al., 2013; Zhang et al., 2014; Ohira et al., 2017; Feng et al., 2018). The GPR 41 and 43 have been renamed as free fatty acid receptor (FFAR) 3 and 2, respectively (Besten et al., 2013). These receptors are widespread in the body, but highly expressed in adipocytes, immune cells and the intestinal tract (Brown et al., 2003; Le Poul et al., 2003; Nilsson et al., 2003). Further, GPR43 (FFAR 2) has a high affinity for propionate and acetate than butyrate, whereas GPR41 (FFAR3) has a high affinity for butyrate and propionate than acetate (Brown et al., 2003; Le Poul et al., 2003). The GPR109A, on the other hand, belongs to the niacin receptor family (Wise et al., 2003; Singh et al., 2014), has high affinity for butyrate as well as  $\beta$ -hydroxybutyric acid, and is highly expressed in intestinal epithelial cells, immune cells and adipocytes (Taggart et al., 2005; Koh et al., 2016; Feng et al., 2018).

The physiological effects of the major SCFA (butyrate, propionate, and acetate) have been the object of extensive reviews in pigs (Jha and Berrocso, 2015; Liu, 2015; Knudsen et al., 2016). Briefly, SCFA contributes to the dietary energy supply once absorbed and reports (Yen, 1997) suggest SCFA can supply up to 28% of the pig's maintenance energy requirement. Further, butyrate is used by the colonocytes as an energy source, whereas propionate and acetate are largely absorbed and transported to the liver. In the liver, propionate is used for gluconeogenesis, whereas most of the acetate is transported to muscles and other peripheral tissue for metabolism (Slavin, 2013; Knudsen et al., 2016). The SCFA have also been reported to stimulate epithelial proliferation and barrier function, regulate the immune system and satiety (Liu, 2015). In pigs, infusion of SCFA into the distal ileum enhanced intestinal barrier function by increasing the abundance of tight junction proteins (TJP-1, OCLDN, and CLDN-1), and mucins (MUC-1), while decreasing the abundance of pro-inflammatory cytokines (IL-8, IL-1 $\beta$ , and TNF- $\alpha$ ) in the ileum and colon (Diao et al., 2017). Further, in the study by Diao et al. (2017) distal ileal infusion of SCFA also increased the expression of GPR43 (FFAR2), GPR41 (FFAR3) and intestinal growth-related genes (IGF-1, IGF-1R, EGF, GLP-2, and GLP-2R). Activation of these receptors has also been associated with the release of PYY and GLP-1 associated with slowed intestinal transit and regulation of appetite.

Butyrate has been reported to be the most important regulator of tight junction proteins, gut hormones and inflammatory responses (Morrison and Preston, 2016; Ohira et al., 2017). However, the above-mentioned studies suggest that propionate independently play a significant role in modulating the immune system and satiety as propionate is the main signaling molecule for both FFAR2 and FFAR3 (Brown et al., 2003; Le Poul et al., 2003). Acetate can also induce expression of PYY (Samuel et al., 2008), antimicrobial peptide LL37 (Ashida et al., 2011) or bind GPR 41 and GPR43 on the intestinal immune cells and to regulate inflammation (Wu et al., 2017; Li et al., 2018). Recently, Feng et al. (2018) reported that sodium butyrate can ameliorate diarrhea symptoms and increase tight junction proteins (CLDN-3, OCLDN, and ZO-1) expression in the colon of weanling pigs' receptor GPR109A. This study implicates butyrate is the main signaling molecule for GPR109A. However, excessive SCFA production has been implicated in neonatal necrotizing enterocolitis (Lin, 2004), while high colonic butyrate concentrations have been implicated in carcinogenesis (Lupton, 2004; Morrison and Preston, 2016). Nonetheless, the beneficial effect of SCFA on intestinal epithelia will depend on factors such as amount, the age of animal and interaction with dietary fat (Lupton, 2004).

Protein from exogenous and endogenous sources that escape enzymatic digestion can be fermented by the gut microbiota (Macfarlane and Macfarlane, 1995) and this occurs through oxidative and reductive reactions including deamination and decarboxylation. In pigs, protein fermentation occurs at the more distal part of the GIT, although significant fermentation can also occur in the proximal GIT (Pieper et al., 2016). Availability of undigested protein for fermentation favors the proliferation of proteolytic bacteria belonging to the *Clostridium*, *Bacteroides*, *Propionibacterium*, *Streptococcus*, *Escherichia-Shigella* (Dai et al., 2011; Boudry et al., 2013; Niu et al., 2015). Most of the proteolytic bacteria have been implicated in diarrhea and GIT dysbiosis (Opapeju et al., 2009; Brower-Sinning et al., 2014) and thus explain in part why protein fermentation is deleterious for pig gut health even later in life (Boudry et al., 2013).

Protein fermentation in the GIT results in the production of SCFA and other metabolites including, ammonia, hydrogen sulfide, phenols and indoles, and biogenic amines. Some of these metabolites are believed to exert various effects on the intestinal epithelial cells, which can be beneficial or deleterious as discussed below.

The SCFA acetate, propionate, and butyrate can also be produced through deamination of several amino acids (AA) including threonine, lysine, glycine, alanine, and glutamate (Macfarlane and Macfarlane, 1995; Smith and Macfarlane, 1997). However, the branched chain fatty acids (BCFA) isobutyrate, isovalerate, and 2-methylbutyrate originate exclusively from deamination of the branched-chain AA valine, leucine, and isoleucine, respectively by bacteria such as *Bacteroides* spp., *Propionibacterium* spp., *Streptococcus* spp., and *Clostridium* spp. (Macfarlane and Macfarlane, 1995; Smith and Macfarlane, 1997). The metabolism and physiological effect of BCFA on intestinal epithelial cells is not well known. However, no direct adverse effect of the BCFA on the intestinal epithelia have been reported, while isobutyrate has been reported to serve as a source of energy for colonocytes under conditions of defective butyrate oxidation or low butyrate availability (Jaskiewicz et al., 1996). Furthermore, in rat adipocytes, BCFA (isobutyrate and isovalerate) inhibited cAMP-mediated lipolysis and insulin-stimulated de novo lipogenesis (Heimann et al., 2016) suggesting their role in regulating lipid and glucose metabolism and signaling in adipocytes.

Ammonia is produced through deamination of AA by gut microbiota and to a lesser extent through urea hydrolysis by urease activity; the latter occurs as a result of urea redirecting from the systemic circulation into the large intestine (Mosenthin et al., 1992). Ammonia concentrations increase gradually from the proximal to the distal part of the hindgut and are highest in the large intestine (Blachier et al., 2007). Ammonia is considered mostly as a toxic metabolite due to reports from in vivo and in vitro studies. For instance, perfusion of rat colon, in situ, with ammonium chloride resulted in disorganized and sloughing of the epithelial cell leading to histological damage, loss of mucus, and significant DNA losses (Lin and Visek, 1991). However, ammonia chloride did not induce epithelial cell necrosis, in pig isolated colonic crypt (Leschelle et al., 2002). Ammonia has also been reported (Darcy-Vrillon et al., 1996; Cremin et al., 2003) to inhibit butyrate activation and (or) butyrate oxidation in mitochondria, while reducing acetate and propionate oxidation (Cremin et al., 2003) and thereby interfere with the oxidative metabolism of colonocytes. In pig colonic tissue and Caco-2 cells, ammonia decreased the expression of MCT-1 even when higher butyrate concentrations were present and increased the expression of IL-8 and TNF- $\alpha$  (Villodre Tudela et al., 2015). The toxic effect of ammonia on intestinal epithelial cells appears to be mediated by its

negative effect on colonic butyrate uptake and promotion of pro-inflammatory signaling (Pieper et al., 2016).

Hydrogen sulfide ( $H_2S$ ) is produced by sulfur-reducing bacteria through fermentation of sulphur-containing AA (such as methionine and cysteine), dietary inorganic sulfate, and sulfur-containing mucins (Gibson et al., 1988; Smith and Macfarlane, 1997; Blachier et al., 2007). There is evidence to suggest that increased exposure to bacterial-derived luminal  $H_2S$  can be potentially deleterious to the intestinal epithelial cells (Linden, 2014). For instance, excessive concentration of  $H_2S$  has been reported to inhibit butyrate oxidation, as well as glutamine and acetate oxidation, in colonocytes leading to a reduction in colonic epithelial cell respiration (Leschelle et al., 2005). The reduction in butyrate oxidation has also been reported to reduce sodium absorption and mucin secretion (Roediger et al., 1993; Jorgensen and Mortensen, 2001). Hydrogen sulfide can also act as an inhibitor of cytochrome c oxidase, the final step in adenosine triphosphate production and thereby impede ATP production and cellular respiration (Cooper and Brown, 2008). In a recent experiment, increased exposure of rat colonocytes to NaHS increased the mRNA levels of hypoxia-inducible factor 1 $\alpha$  (Hif-1 $\alpha$ ) along with inducible nitric oxidase synthase and IL-6 (Beaumont et al., 2016). Therefore, luminal  $H_2S$  can act as a causative factor for chronic intestinal inflammation and intestinal cancers. On the other hand,  $H_2S$  has been reported to have beneficial effects including modulate of inflammation and participate in the resolution of colitis. (Blachier et al., 2010).

Phenolic and indolic compounds are produced as a result of bacterial degradation of the aromatic AA (Macfarlane and Macfarlane, 1995; Smith and Macfarlane, 1997). Phenylalanine catabolism leads to phenyl-containing compounds (e.g., phenylacetate, phenylpyruvate, and phenyllactate), whereas tyrosine catabolism of leads to hydroxyphenyl-containing compounds (e.g., hydroxyphenyl acetate, hydroxyphenyllactate, and hydroxyphenylpyruvate) as well as phenols and p-cresol (Smith and Macfarlane, 1997). Tryptophan fermentation generates indole and skatole (Smith and Macfarlane, 1997).

Phenols are widely considered to have negative effects on the host and high levels of p-cresol in the urine have been associated with intestinal inflammation and colon cancer (Jacobs et al., 2009). Exposure of colonic epithelial cells to phenols significantly impaired viability (Pedersen et al., 2002). Additionally, incubation of Caco-2 cells with phenol decreased transepithelial resistance and increased permeability (Hughes et al., 2008; McCall et al., 2009). Exposure to phenol also delocalized CLDN-1 and ZO-1 from tight junctions to the cytosol, suggesting that phenol can affect the lipid bilayer of the cell membrane and thus destabilize the tight junction proteins that regulate epithelial barrier function (McCall et al., 2009)

Indoles, on the other hand, have been reported to have positive effects on the intestinal epithelial cells. For instance, exposure of the human cell line HCT-8 to indoles increased the expression of several genes involved in mucosal barrier strengthening (including, CLDN-3, ZO-3, TJP-1, and TJP-3) and mucin production (MUC-1, MUC-3, MUC-13, MUC-20) (Bansal et al., 2010). The authors also reported that indole decreased TNF- $\alpha$ -mediated activation of NF- $\kappa$ B, IL-8 gene expression, and pathogenic E. coli attachment to the cells while increasing the gene expression of anti-inflammatory IL-10. Administering indole orally enhanced the mRNA expression of the tight junction- and adherens junction-associated molecules in colonic epithelial cells of germ-free mice (Shimada et al., 2013), suggesting improved intestinal barrier function. Indole has also been

reported to modulate oxidative stress, and GLP-1 secretion by intestinal enteroendocrine L cells and thus can impact metabolic disease, such as type 2 diabetes (Chimerel et al., 2014). Currently, indole and derivatives of indoles are viewed as putative anti-virulence compounds against antibiotic resistance due to its ability to inhibit quorum sensing and virulence factor production (Lee et al., 2015).

Amines are low molecular nitrogenous organic polycations, in which up to 3 hydrogens in ammonia are replaced by alkyl or aryl groups (Shalaby, 1996). Polyamines (putrescine spermidine, and spermine) and amines (agmatine, cadaverine, tyramine and histamine) found in the gut lumen originate from 4 sources: dietary origin, endogenous secretion (synthesis via ornithine decarboxylase), colonic microflora metabolism, and desquamated colonocytes in the course of epithelium renewal (Blachier et al., 2007). However, decarboxylation of AA is the most common mode of synthesis of amines and are referred to as biogenic when they are formed through bacterial decarboxylation of AA (Shalaby, 1996). Polyamines (spermidine, spermine, and putrescine) have been widely studied due to their essential role in mammalian intestinal mucosal growth (Löser et al., 1999; Mitchell et al., 2002), malignant cell transformation, and ability to interfere with numerous cellular processes such as DNA, RNA and protein synthesis (Blachier et al., 2007). Histamine also has numerous physiological effect on the intestine including goblet cell secretion, cell proliferation and differentiation, and smooth muscle contraction (Gaskins, 2001). Histamine alters the dendritic cell response to microbes by enhancing IL-12 secretion via H1-receptor, whereas H2-receptor activation promotes IL-10 secretion and inhibits IL-12, TNF- $\alpha$ , and IL-23 secretion (Smolinska et al., 2014). Thus, histamine can have a pro-inflammatory or anti-inflammatory effect depending on which histamine receptor is activated (Smolinska et al., 2014). In porcine colon, histamine acts directly through H2-receptors to induce fluid secretion (Ahrens et al., 2003)

In weanling pigs, high colonic concentrations of putrescine, spermidine, and histamine were associated with increased mRNA expressions of marker genes associated with cell turnover (proliferating cell nuclear antigen, PCNA), pro-inflammatory response (IL-1 $\beta$ , IL-6, MUC-1, MUC-2, MUC-20), and anti-inflammatory response (IL-10 and TGF- $\beta$ ) (Pieper et al., 2012) suggesting that biogenic amines regulated colonic mucosal response without causing actual systemic or local inflammation. Clearly, the contribution of biogenic amines to gut health problems is unknown. However, in a more recent study, piglet colonic mRNA expressions of MCT 1 was negatively correlated with putrescine (Villodre Tudela et al., 2015), suggesting that luminal putrescine could reduce the uptake of butyrate into intestinal epithelial cells.

## **Summary and Conclusion**

There is strong evidence that the GIT microbiota contributes significantly to the development and function of the gastrointestinal tract. These contributions affect the primary function of the GIT including both nutrient assimilation and barrier function. Host animals have evolved a number of mechanisms to sense diet-dependent and diet independent changes in microbial community membership and metabolic activity, respectively. Diet independent changes primarily reflect changes in microbial composition which rely on receptor-mediated detection of microbial components (MAMPs) and the site of detection (host or luminal side of the epithelial barrier) to differentiate friend (probiotic or commensal) from foe (pathogens). That both commensal and pathogens are “sensed” by the hosts suggests controlling commensal microbial representation in the GIT could

improve host immune response on exposure to pathogens. Whether environmental influences affecting barrier function in the neonate can impact the recognition of a microorganism as friend or foe and the nature of the response deserves investigation. Diet-dependent changes primarily reflect microbial fermentation of feed ingredients which can result in a plethora of metabolites which are sensed by the host using a variety of pathways. Fermentation of fibre is generally considered to generate beneficial metabolites whereas fermentation of protein generates metabolites with mostly toxic effects. However, commensurate with the extensive fermentation capacity of the complex microbiota, there is a vast capacity to produce metabolites with beneficial or harmful effects that have not been fully appreciated. Understanding the mechanism sensing both changes in microbial composition and their metabolites is a key component in promoting gut health.

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# **Microbial Endocrinology: Why the Evolutionary-based Integration of Microbiology and Neurobiology Matters in the Examination of the Intersection of Animal Nutrition and Epigenetics**

## **Endocrinologie microbienne : pourquoi l'intégration de la microbiologie et de la neurobiologie basée sur l'évolution compte dans l'examen de l'intersection de la nutrition animale et de l'épigénétique**

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### **Abstract**

Advancements in understanding the importance of bi-directional communication between host and microbiome stand to redefine the relationship between animal nutrition and epigenetics. Microbial endocrinology, the intersection of neurobiology and microbiology, has demonstrated that neuroendocrine-immune axes are major hubs of host-microbiome crosstalk, and potentially programmable by nutrition. That host and microbiome can possess the same genes and produce structurally identical neurochemicals highlights a microbial endocrinological role for epigenetic regulation of host-microbe neuroendocrine-immune crosstalk. Animal foods also contain many of the same neurochemicals found in the host and microbiota. Thus, all three elements, host, microbe and nutrition, interact. Host and bacterial metabolites may regulate expression of host or bacterial neuroendocrine genes to epigenetically affect such interaction. Discussion will also include how neurochemical-containing foods fed to animals can affect both animal and microbiota. As environmental stressors can affect gut health, this paper will additionally examine how microbial endocrinology can help frame investigations seeking to isolate the role of bacterial metabolites in mediating protective epigenetic effects on animal gut health in real-world conditions. Finally, the effects of dietary micronutrients on gut epithelial DNA methylation and intestinal inflammation will be contextualized within microbial endocrinology and relevance to animal health. As we are at the forefront of understanding nutritionally-driven epigenetic plasticity of animal neuroendocrine-immune systems within the context of host-microbiome interaction, microbial endocrinology stands to provide strong conceptual and methodological frameworks to identify novel targets at the intersection of animal nutrition and epigenetics of value to the animal production industry.

## Résumé

Les avancées dans la compréhension de l'importance de la communication bidirectionnelle entre l'hôte et le microbiome permettent de redéfinir la relation entre la nutrition animale et l'épigénétique. L'endocrinologie microbienne, l'intersection de la neurobiologie et la microbiologie, a démontré que les axes neuroendocriniens-immuns sont des carrefours majeurs du dialogue hôte-microbiome, potentiellement programmables par la nutrition. Que, du point de vue évolutif, l'hôte et le microbiome puissent posséder les mêmes gènes et produire des neurohormones structurellement identiques met en évidence un rôle endocrinologique microbien pour la régulation des miARN épigénétiques dans le dialogue neuroendocrinien-immun hôte-microbe. En outre, les aliments des animaux contiennent plusieurs des mêmes éléments neurochimiques que l'on trouve dans l'hôte et le microbiote. Cela signifie que tous les trois éléments – l'hôte, le microbe et la nutrition – interagissent ensemble. Qui plus est, l'hôte et les miARN bactériens peuvent réguler l'expression des gènes de l'hôte ou des gènes neuroendocriniens bactériens pour influencer cette interaction au niveau épigénétique. La discussion inclura aussi comment les aliments contenant des éléments neurochimiques donnés aux animaux peuvent affecter tant l'animal que le microbiote. Comme les stressseurs de l'environnement affectent aussi la santé de l'intestin, cette présentation examinera comment l'endocrinologie microbienne peut aider à encadrer les investigations cherchant à isoler le rôle des métabolites bactériens dans la médiation des effets épigénétiques protecteurs sur la santé de l'intestin de l'animal, en conditions réelles. Enfin, les effets des micronutriments alimentaires sur la méthylation de l'ADN épithélial dans l'intestin seront mis en contexte dans l'endocrinologie microbienne et la pertinence à la santé animale. Alors que nous sommes à l'avant-garde pour comprendre la plasticité épigénétique nutritionnelle des systèmes neuroendocriniens-immuns des animaux dans le contexte de l'interaction hôte-microbiome, l'endocrinologie microbienne peut offrir des cadres de travail conceptuels et méthodologiques servant à identifier de nouvelles cibles concernant l'intersection de la nutrition animale et l'épigénétique qui seront utiles à l'industrie de la production animale.

## Introduction

Advances in nutrition and genetics have led to significant improvements across virtually all animal production industries. Currently, the nutritional and genetic plasticity of performance traits that play economically-important roles in poultry, swine, cattle, and other animals is being re-defined with increased recognition of host-microbiome interaction in mediating animal health and physiology. Microbial endocrinology, which is the union of microbiology and neurobiology, is the study of neurochemicals as an evolutionary-based bi-directional language between microbe and host (Lyte, 2014). That microbes produce and recognize physiologically-important neurochemicals that are *structurally-identical* to those synthesized by the host, and that animal feeds contain neurochemicals and precursors, underscores the importance of integrating microbial endocrinology into strategies to improve the health of poultry (Cogan et al., 2007, Aroori et al., 2014, Villageliu and Lyte, 2017), swine (Oneal et al., 2008, Hegde et al., 2009, Anderson and Armstrong, 2006, Lyte and Lyte, 2019), ruminant (Vlisidou et al., 2004, Lyte et al., 2018), and other animals (Lyte, 2016a, Gervasi Stephanie et al., 2016).

Within the framework of microbial endocrinology, nutrition must not be thought of as solely feeding the animal but also as supplying both host and microbiota with neurochemicals and

precursors useable in a bi-directional neuroendocrine dialogue influential of disease pathogenesis, gut health, nutrient partitioning, immune function, and other economically-relevant animal traits. As key host genes involved in the synthesis of neurochemicals are thought to have originated via lateral gene transfer from prokaryotes (Iyer et al., 2004), it should not be surprising that the same environmental stimuli, such as nutrition, represent epigenetic avenues affective of both microbial and host neuroendocrine-immune gene regulation. Indeed, microbial and host synthetic pathways can utilize the same amino acids to produce identical neurochemicals (Lyte, 2018). It is important to recognize that some neurochemicals play pro- and anti-inflammatory roles (Gershon, 2012), and certain pathogens are even known to utilize host stores of amino acids during the first 24 hours of infection (Abromaitis et al., 2009). This is important as changes in the neuroendocrine environment of the gut, for example, following stress can cause the upregulation of microbial virulence gene expression. Hence, microbial endocrinology stands to offer a testable framework by which diet may act in an epigenetic-manner to protect against the deleterious effects of stress-related disease. Additionally, bacterial metabolism of what the host has eaten can also result in the production of microbial metabolites that regulate host neuroendocrine-immune gene expression locally within the gut and systemically. Animal nutrition therefore represents a tailorable epigenetic strategy by which microbial endocrinology can be directly applied to animal production.

## **Overview of Manuscript Structure**

Host-microbe neuroendocrine crosstalk is sensitive to a vast number of different environmental factors, some of which have been described to mediate epigenetic changes. As microbial endocrinology emphasizes the bi-directional nature of interkingdom neuroendocrine communication, such epigenetic changes must, too, be viewed not as isolated effects on host *or* microbe but instead as potentially impactful on both host *and* microbe. Butyrate, a short chain fatty acid (SCFA), is used as a prototypical example throughout this manuscript to illustrate the complex nature of an epigenetic molecule in how it can impact host, microbe, and the dynamic by which host and microbe interact with particular attention towards host health. A major reason why butyrate was chosen in this manuscript is that it is very well studied not only in rodents and humans but also in food production animals including poultry, swine, and cattle—also of note, SCFA, including butyrate, have been studied in mediating production traits in economically-important animals thereby allowing animal producers and industry to appreciate the epigenetic role of a commercially-applicable molecule in the context of host-microbe interaction.

This manuscript will lead with the multiple epigenetic effects that one molecule can exert, with focus given towards how beneficial outcomes must be weighed in light of potentially deleterious effects. From there, nutritional strategies which seek to emphasize an epigenetic effect will be examined through the lens of microbial endocrinology, with suggestions on how to approach this topic. Lastly, attention is given to how nutritional epigenetics may alter host-microbe neuroendocrine crosstalk and how this may affect stress and disease.

## **Epigenetic Tailoring of the Neuroendocrine System: Saw or Scalpel?**

At the outset of this paper, it must be emphasized that the same nutritive or non-nutritive component of animal feeds can exert potentially desirable and undesirable epigenetic effects on the animal. As very little has been done in the way of studying how these complementary or contrasting epigenetic

effects together affect animal health, or that of the microbiota and how it may be influential of host-microbe interaction, microbial endocrinology offers a conceptual framework by which the epigenetic effects of nutrition may be understood as bi-directional and comprehensive.

One such example is butyrate, a SCFA found endogenously in food, germ-free animals (i.e. animals that are sterile and have no microbiota) gain butyrate through diet (Hoverstad and Midtvedt, 1986) and, to a greater extent, as the product of microbial fermentation in the gastrointestinal tract of poultry (Onrust et al., 2015), swine (Levine et al., 2013) as well as the rumen (Sutton et al., 2003). A large number of studies have demonstrated beneficial effects of butyrate on gut and/or rumen health of food production animals (Bedford and Gong, 2018). As a histone deacetylase inhibitor, butyrate may exert some of its protective effects on the gut epithelium via epigenetic mechanisms in poultry (Sunkara et al., 2011) and swine (Zeng et al., 2013), as well as cause diverse transcriptomic changes in the rumen epithelium (Baldwin et al., 2018).

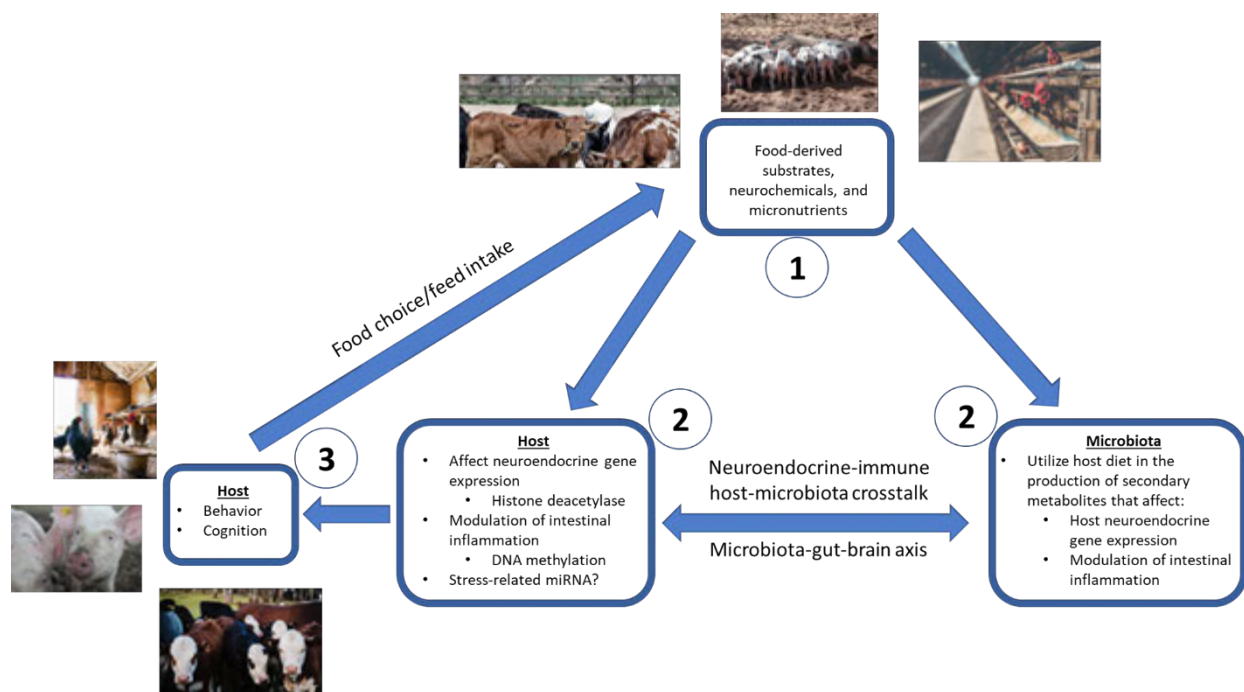
It is therefore noteworthy that butyrate has also been reported to influence animal locomotor activity (Moretti et al., 2011) and activate the hypothalamic-pituitary-adrenal (HPA)-axis (Gagliano et al., 2014), a major neuroendocrine system present in all animals that results in the production of corticosterone in birds as well as rodents, and its analogue cortisol in swine and ruminants (cortisol and corticosterone are hereafter referred to as CORT). Aside from the beneficial role of CORT in mediating adaptation to stress, CORT has been well-described to negatively impact economically-important measures such as growth rate and feed efficiencies in food production animals (Mormede et al., 2011). As microbial endocrinology has revealed neuroendocrine systems to serve as hubs of host-microbe crosstalk, that butyrate affects the host HPA-axis highlights the need to look comprehensively at how such epigenetic activation may feedback onto the microbiota to modify host-microbe interaction relevant to host health. This is especially important considering the in-feed addition of butyrate has been studied, with mixed results, in growth promotion (Weber and Kerr, 2008), as an antibiotic alternative (Walia et al., 2016, Fernandez-Rubio et al., 2009), in ruminal development (Niwinska et al., 2017), and in other contexts (Bedford and Gong, 2018). As such, a major question, for which microbial endocrinology is poised to answer, remains: Are the beneficial putatively epigenetic effects of butyrate occurring alone, or is butyrate also effecting meaningful epigenetic changes in other places, such as the neuroendocrine-immune axes?

## **Nutritional Epigenetics and Dietary Neurochemicals: An Emerging Picture**

Microbial endocrinology-based mechanisms of bi-directional neuroendocrine interkingdom communication in which butyrate has a described, but underexplored, role include not only CORT and glucocorticoids but also monoamines and other signaling molecules (Neuman et al., 2015). This is an important point to consider because the neuroactive potential of animal nutrition in mediating host-microbe interaction has not been examined in light of potentially competitive epigenetic influences.

A prominent example of this complex epigenetic picture is gastrointestinal inflammation, which is an area of investigation important to all food production animals. The serotonergic system in the gut, which has been described to play both pro-inflammatory and anti-inflammatory roles, is strongly mediated by host-microbe interaction and sensitive to dietary epigenetic factors. Tryptophan hydroxylase (TPH)-1, found in the intestinal epithelium, is a key rate-limiting host

enzyme in the production of serotonin from the amino acid tryptophan. Butyrate has been reported to influence TPH-1 gene expression in the gut to alter serotonin in the mucosa (Reigstad et al., 2015). From the standpoint of animal nutrition, tryptophan as well as serotonin (Waalkes et al., 1958, Ly et al., 2008) in the diet is likely to be accompanied by other factors including micronutrients. For example, zinc, a dietary micronutrient, was recently demonstrated to alter inflammation in the chick mucosa via epigenetic routes of DNA methylation and histone acetylation (Li et al., 2015a). To further complicate the picture, butyrate also appears to mediate gut barrier function via epigenetic induction of IL-10, an anti-inflammatory cytokine, gene expression (Zheng et al., 2017) as well as alter intestinal macrophage function through histone deacetylase inhibition (Chang et al., 2014). Hence, the prospect of nutritional epigenetic regulation of gastrointestinal inflammation is incredibly complex and, for the animal producer, a targeted nutritional epigenetic approach is likely not possible. Instead, context-specific approaches should be designed where one modifiable dietary factor may tip the scale in favor of, for example, reduced gut inflammation. Design of diets based on the epigenetic potential of feed components that contain substrates fermentable into SCFA, such as butyrate (Li et al., 2018), or convertible into neurochemicals should be considered within the framework of microbial endocrinology as these factors can potentially alter host-microbe crosstalk both locally in the gut, and within the central nervous system (CNS) to alter feeding behavior (Figure 1).



**Figure 1.** Hypothetical routes by which (1) animal nutrition mediates (2) epigenetic bi-directional host-microbiota interactions along neuroendocrine-immune axes in food production animals. Feed components and microbial fermented products, such as short chain fatty acids, may (3) affect host locomotor and feeding behaviors.

## Nutritional Epigenetics and Neurochemicals: A Role in Stress, Disease, and Health Management?

The neuroendocrine axes which mediate an animal's response to stress also represents an important bi-directional interkingdom pathway of host-microbe interaction. Indeed, within the field of microbial endocrinology, catecholamines, such as norepinephrine, have been demonstrated to strongly influence microbial infection and pathogenesis (Lyte, 2016b) while others, for example dopamine and dopaminergic agonists play anti-inflammatory roles (Gough, 2015) and may hold promising therapeutic potential in gut-inflammation related disease (Oehlers et al., 2017). Produced not only in the adrenal medulla, but also in the gut (Asano et al., 2012) and the lung (Dickson et al., 2015), catecholamines have been shown to affect pathogens important to poultry (Aroori et al., 2014, Xu et al., 2015), swine (Toscano et al., 2007, Li et al., 2015b), and cattle (Pullinger et al., 2010). Likewise, dopaminergic agonists have shown substantial anti-inflammatory activity in currently incurable inflammatory gastrointestinal diseases, such as irritable bowel disease (Oehlers et al., 2017). It is therefore of note that nutrition may epigenetically-affect genes involved in catecholamine synthesis. Tyrosine hydroxylase (TH) is the first and rate-limiting step in catecholamine synthesis. SCFA, including butyrate, have been demonstrated to affect TH gene expression, in part, through epigenetic mechanisms (Kim et al., 2003, Lenartowski and Goc, 2011). As butyrate also affects the HPA-axis, it is interesting to speculate an epigenetic role of SCFA in host stress response and inflammation and whether this may feedback onto the microbiota. Indeed, CORT produced in the adrenal cortex can accumulate in the adrenal medulla, and, in a concentration-dependent fashion, stimulate catecholamine synthesis. Hence, it would be interesting to examine whether potential epigenetic effects of butyrate on different stress pathways ultimately intersect and influence each other to dampen inflammation, potentially via dopamine production

When considering nutritional epigenetics in animal production, the epigenetic effects of SCFA on stress pathways, or stress-related gene expression, should also be considered to potentially affect downstream targets, including influencing microbe-microbe interaction relevant to host health. As TH synthesizes L-3,4 dihydroxyphenylalanine (L-dopa) from the amino acid tyrosine, it is highly relevant that microbes can synthesize dopamine from L-dopa. Recently, *Enterococcus faecium* was first described to produce dopamine when supplied with the precursor L-dopa (**Table 1**) (Villageliu and Lyte, 2018). *E. faecium* is a probiotic used in poultry (Cao et al., 2013), swine (Pollmann et al., 2005), and cattle (Qadis et al., 2014). As mentioned earlier, in addition to its constitutive presence in normal diets, butyrate is also a major microbial fermentation product. Hence, from a microbial endocrinological epigenetic standpoint it is necessary to consider whether diets which supply fermentable substrates to result in the production of butyrate can epigenetically-drive TH expression to influence the production of L-dopa, providing substrate for the production of dopamine that can play anti-inflammatory roles in the gut to positively affect host health.

**Table 1.** Heterogeneity in dopamine production across varying strains of *E. faecium*<sup>1</sup>.

<u>Strain</u>	<u>Source</u>	<u>L-DOPA consumed µg/ml</u>	<u>Dopamine produced µg/ml</u>	<u>Conversion Efficiency (%)</u>
ML1081	Fortiflora	146.2 (+/- 0.68)	87.8 (+/- 0.9)	63.1
ML1082	Probios	176.5 (+/- 0.52)	133.7 (+/- 1.4)	96.1
ML1085	Canine urine	126.8 (+/- 1.8)	77.9 (+/- 0.7)	56.0
ML1086	Canine incision	176.2 (+/- 0.7)	105.0 (+/- 1.1)	76.2
ML1087	Feline urine	11.7 (+/- 1.8)	9.7 (+/- 0.4)	7.0
ML1088	Avian yolk sac	64.2 (+/- 2.8)	36.9 (+/- 1.5)	26.5
ML1089	Canine bile	161.3 (+/- 1.9)	104.5 (+/- 1.9)	75.1

<sup>1</sup>Data from (Villageliu and Lyte, 2018).

## Conclusions and Future Directions

We are at the forefront of understanding nutritional epigenetics in the context of host-microbe interaction. Microbial endocrinology stands to provide a conceptual framework in which the epigenetic plasticity of host and microbial genes may be strategically targeted using nutrition. As food animal production traits are increasingly contextualized within host-microbe crosstalk, future research which seeks to understand an epigenetic role of nutrition must approach diet as feeding both host and microbe. Furthermore, epigenetic effects should not be expressly viewed as isolated events at specific genes, either in the host or in the microbe. Instead, there is significant likelihood for epigenetically-active metabolites or feed components, which are neurochemically-recognized by both microbe and host, to likely have multiple effects at diverse genetic sites in both host and microbe. Microbial endocrinology posits that neuroendocrine-immune axes are likely targetable sites in which future investigations may explore how nutritional epigenetics may affect host-microbe interaction to the benefit of the animal production industries

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## **How Can Early Social Environment Impact Epigenetics, Health and Inform Choices**

### **Comment l'environnement social précoce influence l'épigénétique, la santé et éclaire les choix**

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#### **Abstract**

While the gene sequence contains the “operating system” of the genome epigenetic processes code the “Applications” that program and functionalize the DNA in time, context and space. The biochemical processes involved in epigenetic “programming” have been intensively studied in the last 5 decades and they include covalent modifications of the DNA sequence itself by DNA methylation and further oxidations of the methyl moiety, covalent modifications of histones and noncoding RNAs. Epigenetic programming regulates “cell differentiation” and specification during embryonal development. However, data that emerged in the last decade provides evidence for “epigenetic programming” by experience, particularly social experience. We will review data from rodents and nonhuman primates showing that maternal care affects long term phenotypes which are potentially mediated by DNA methylation. The impact of early life experience is not limited to the brain and affects the immune system as well as other tissues suggesting that social experience has a “system wide” impact on the developing animal. Human data from the Quebec ice storm of 1998 provides further evidence for the impact of early life stress on DNA methylation and its mediating effects on metabolic, immune and behavioral phenotypes. How does social experience which is registered in the brain impact such a wide panel of tissues? We tested the hypothesis that the glucocorticoid receptor might be mediating the impact of stress on DNA methylation. We show broad changes in DNA methylation in the placenta in pups with a heterozygous knockout of the *nr3c1* gene encoding the glucocorticoid receptor.

#### **Résumé**

Alors que la séquence génétique contient le « système d'exploitation » des processus épigénétiques génomiques, les « applications » codifient les programmes et les fonctions de l'ADN dans le temps, le contexte et l'espace. Les processus biochimiques participant à la « programmation » épigénétique ont fait l'objet d'études intensives dans les cinq dernières décennies et elles incluent des modifications covalentes de la séquence d'ADN elle-même par la méthylation de l'ADN et les oxydations plus avancées du groupement méthyle, les modifications covalentes des histones et des ARN non codants. La programmation épigénétique régule la « différenciation des cellules » et la spécification pendant le développement de l'embryon. Cependant, les données qui sont apparues depuis une dizaine d'années prouvent la « programmation épigénétique » par l'expérience,

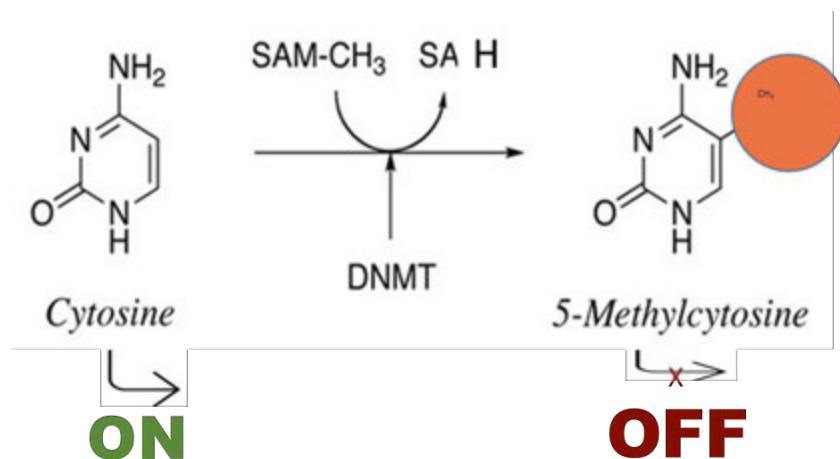
notamment l'expérience sociale. Nous examinerons des données sur les rongeurs et sur les primates non humains montrant que les soins maternels touchent les phénotypes à long terme, qui sont potentiellement médiés par la méthylation de l'ADN. L'impact de l'expérience précoce ne se limite pas au cerveau et affecte le système immunitaire ainsi que d'autres tissus, ce qui donne à penser que l'expérience sociale a une incidence à « l'échelle du système » chez l'animal en développement. Les données humaines provenant de la tempête de verglas au Québec, en 1998, donnent d'autres preuves de l'impact du stress précoce sur la méthylation de l'ADN et des effets de la médiation sur les phénotypes métaboliques, immuns et comportementaux. Comment l'expérience sociale, qui est enregistrée dans le cerveau, affecte-t-elle une si grande zone de tissus ? Nous avons testé l'hypothèse que le récepteur de glucocorticoïdes pourrait médier l'impact du stress sur la méthylation de l'ADN. Nous montrons d'importants changements dans la méthylation de l'ADN dans le placenta des chiots avec inactivation hétérozygote du gène *nr3c1* encodant le récepteur de glucocorticoïdes.

### **Early Life Experience has a Long-term Effect on the Phenotype; What is the Mechanism?**

A long life of evidence has established that experience and particularly early life experience has a long term impact on the phenotype. Interestingly, not only chemical exposure such as exposure to xenobiotics or dietary intake influence phenotypes later in life, but also social exposures early in life have a long-term impact on both physical and mental health. What are the mechanisms that mediate between these exposures and long-term programming of phenotype? We will discuss the emerging hypothesis that epigenetic programming mediates between exposures and experience and the DNA (Szyf, 2009).

### **What is DNA Methylation?**

Epigenetics is a combination of several mechanisms that regulate long term gene programming and gene function (Szyf, 2009). Epigenetic programming is responsible for cell type specific programming, enabling a single DNA sequence to express a vast number of different gene expression programs in space and time. DNA methylation is the most proximal epigenetic mark on DNA. It is a covalent modification by methylation of cytosine and adenine moieties in DNA. Cytosine methylation occurs particularly at the CG dinucleotide sequence (Figure 1).



**Figure 1.** The DNA methylation reactions

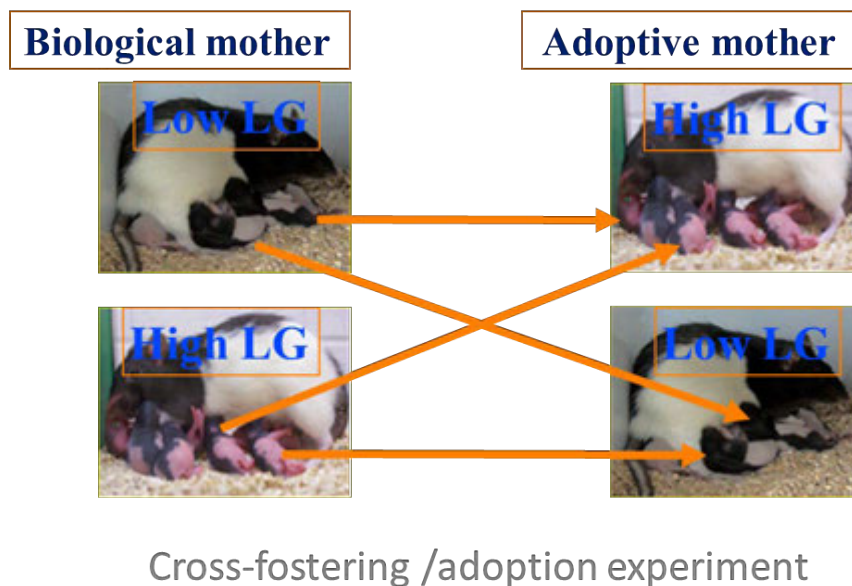
Different CGs are methylated in different tissues, thus there is a cell type specific pattern of DNA methylation. DNA methylation at strategic positions in gene can silence gene expression by either directly interfering with binding of transcription factors or through recruitment of methylated DNA binding proteins such as MeCP2 that in turn recruit other chromatin silencing enzymes. DNA methylation confers upon DNA a cellular identity. These profiles of DNA methylation and other epigenetic programming as well as chromatin modification by histone acetylation, methylation or phosphorylation are generated during embryonal development. DNA methylation is catalyzed by highly regulated enzymes called DNA methyltransferases (DNMT). DNA methyltransferases catalyze the transfer of a methyl moiety from the methyl donor S-adenosylmethionine to the 5' position in the cytosine ring. It was recently discovered that the methyl group on cytosine can undergo further modifications to 5-hydroxymethylcytosines and further oxidized forms such as 5-formyl cytosine and 5-carboxycytosine. This reaction is catalyzed by a different set of enzymes the Tet oxygenases. The biological signal encoded by these higher oxidized forms of 5-methylcytosine is yet unclear (Szyf, 2016). Oxidation of 5-methylcytosine can lead to loss of the methyl group via replication or repair. In summary DNA methylation is a mechanism that confers upon identical sequences of DNA different cellular identities and different functional densities. Thus, phenotypic variation is embedded in DNA without altering the genetic sequence. We are proposing that the same mechanism that is used in development to provide DNA with its cellular identity is utilized for creating an experiential identity (Szyf, 2009).

### **Epigenetic Programming by Maternal Care; Reversal of Epigenetic Programming by Epigenetic Interventions**

The first evidence that a social experience early in life is epigenetically embedded in the DNA and that it can have long term impact on the phenotype came from studies examining the impact on the offspring of differences in maternal care behaviors in rats. There is a natural distribution of maternal care in rats. On both extremes are rats that provide either very High or very Low maternal care. Offspring of rats who received high maternal care during early life exhibit reduced and

controlled stress responsivity during adulthood than rats who received low maternal care. There are other phenotypic differences between these rats. Since one of the characteristic differences in behavior that differentiate rats that received different maternal care is in stress responsivity, we examined differences in epigenetic programming of the Glucocorticoid receptor gene (*NR3C1*) in the hippocampus which is responsible for negative feedback regulation of the hypothalamic pituitary adrenal (HPA) axis by glucocorticoids. Offspring of low maternal care mothers undergo methylation of the *NR3C1* gene in the hippocampus during the first week after birth in response to maternal care, histones around the gene are hypoacetylated (a marker of silent chromatin) and the transcription of the gene is reduced while in rats that received high maternal care the gene is less methylated, histones are hyperacetylated and the transcription of *NR3C1* is increased. These differences in epigenetic programming remain into adulthood. Reduced expression of *NR3C1* in the hippocampus by epigenetic programming could explain the hypersensitive stress response in the low maternal care rat offspring (Weaver et al., 2004; Weaver et al., 2005; Weaver et al., 2007).

To test whether the differences in epigenetic programming and behavior are inherited from the mother are “genetic” or “epigenetic”, triggered by experience, the pups were cross fostered after birth. Offspring of high maternal care mother that were fostered by low maternal care mothers exhibited the phenotype of low maternal care rats whereas offspring of low maternal care mothers that were fostered by high maternal care mothers exhibited the phenotype of high maternal care rats (Figure 2). These experiments provided evidence that the caring mother and not the biological mother transmitted the phenotype.



**Figure 2.** Behavioral programming by the mother is epigenetic not genetic; The fostering mother and not the biological mothers transfers the phenotype. (Meaney et al.,)

Epigenetic marks in difference from genetic changes are potentially reversible since they are introduced to DNA by reversible enzymatic reactions. We therefore tested whether epigenetic programming by maternal care could be reversed in adulthood. Methionine, an amino acid that is a precursor of S-adenosyl methionine the methyl donor of DNA methylation reactions injected

into the brain of offspring of high maternal care mothers increased methylation, reduced histone acetylation, inhibited expression of the *NR3C1* gene and reversed the stress responsivity phenotype of the animals to resemble low maternal care animals. Injection into the brain of offspring of low maternal care of trichostatin A (TSA) histone deacetylase inhibitor that triggers an increase in histone acetylation animals increased histone acetylation, reduced methylation and increased expression of the *NR3C1* gene and reverted the behavior of the animals to resemble the behavior of high maternal care animals (Weaver et al., 2004; Weaver et al., 2005; Weaver et al., 2007).

In summary, early life social experience of exposure to maternal behavior triggers epigenetic changes that program gene function in the brain and establish stable behavioral phenotypes. Nevertheless, these early-life programmed epigenetic differences are reversible by epigenetic pharmacological manipulation.

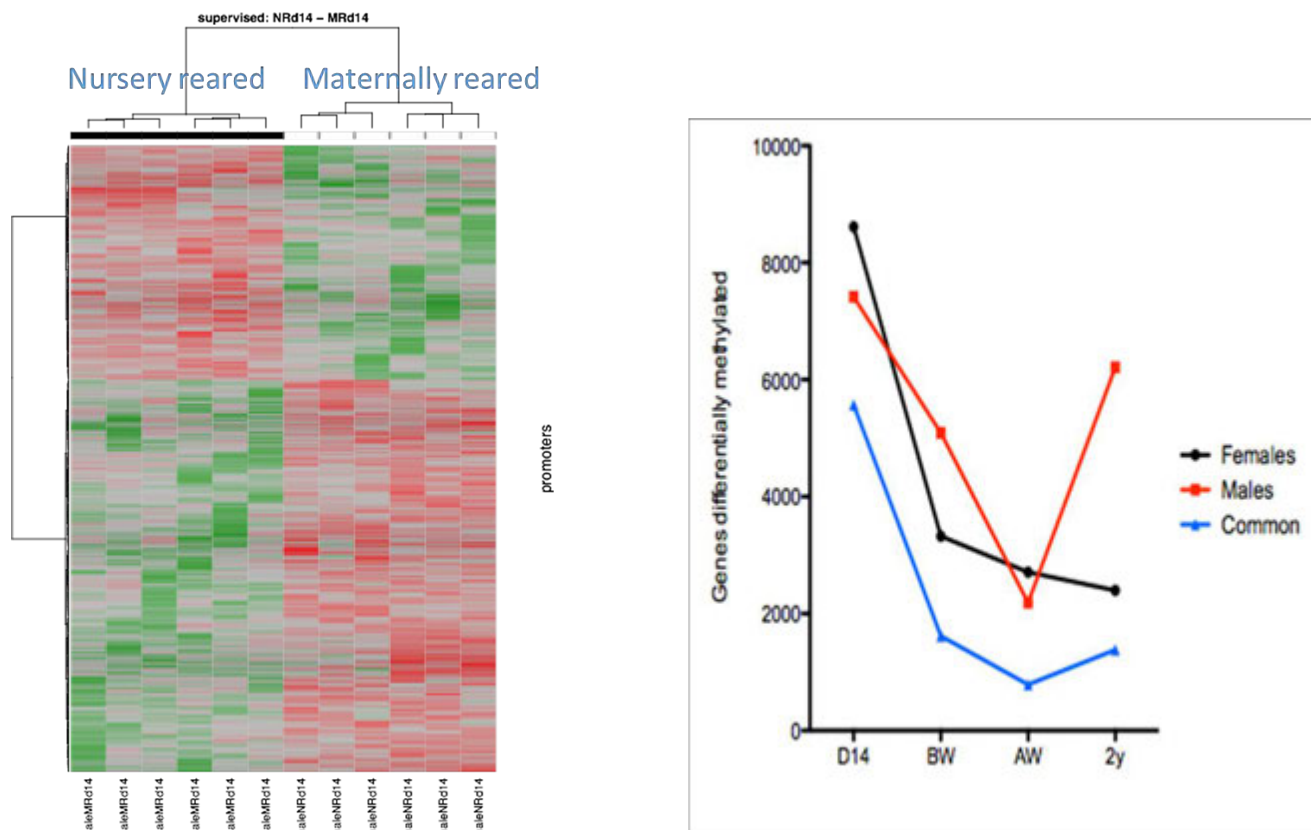
### **Epigenetic Programming by Early Life Experience Affects Many Genes in Several Tissues; Rhesus Macaque Model of Maternal Deprivation**

Maternal care affects expression of hundreds of different genes in the hippocampus as well as the state of methylation of broad genomic regions such as the region containing a cluster of genes in the protocadherin family. Interestingly, the response to early life adversity is evolutionary conserved. The state of methylation of *NR3c1* and protocadherin loci are also altered in humans that were exposed to early life adversity (Suderman, 2012).

We examined whether the response to early life experience is limited to the brain or whether it is also noted in other tissues such as the immune system by examining the pattern of DNA methylation of rhesus macaque monkeys that were exposed to removal of a mother after birth and monkeys that received normal maternal care (Suomi et al.,) after birth. Differences in DNA methylation between the groups when they were adults were noted in both the prefrontal cortex and T cells (Provencal et al., 2012).

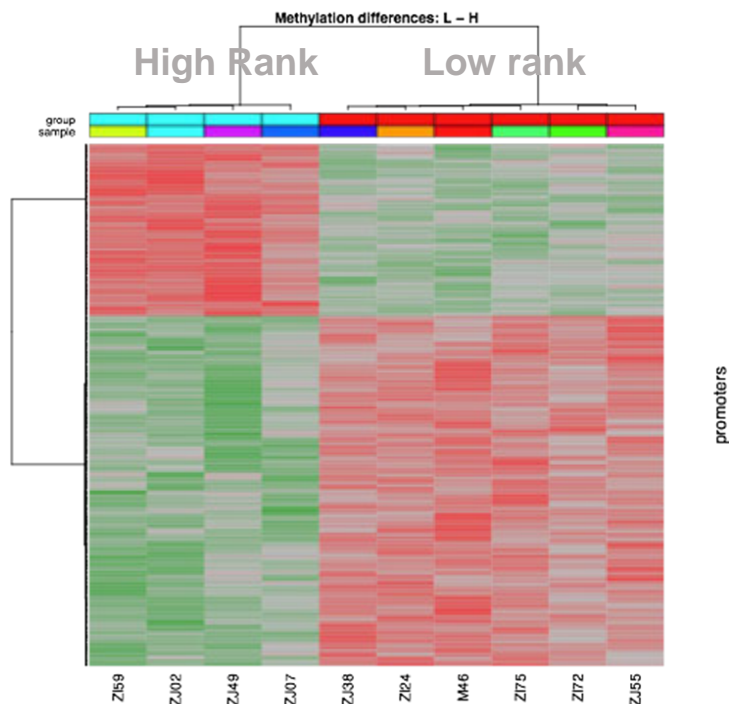
### **Early Life Adversity Affects the Developmental Trajectory of DNA Methylation Patterns in T Cells**

DNA methylation in T cells of the monkeys goes through developmental changes from birth to adolescence which are sex specific. Differences in developmental trajectory of DNA methylation between the maternal reared and nursery reared groups emerge after birth and accompany development (Figure 3). These differences are again sex specific (Massart et al., 2016).



**Figure 3.** Differential DNA methylation associated with early life stress emerges early at d14 in T cells and continue dynamically into adolescence; Early life social status affect DNA methylation profile of the placenta

We determined how early the social behavior of a mother affects the DNA methylation of the offspring. We examined the pattern of methylation of placentae from mothers which were of high and low ranking. Social rank is known to have a large impact on the stress levels and behavior of these monkeys. Large DNA methylation differences were noted between these groups of monkeys (Figure 4); indicating that epigenetic differences that are associated with maternal social status emerge at birth(Massart et al., 2017).



**Figure 4.** Differential DNA methylation in the placenta associated with social rank differences in mothers in rhesus monkeys (Suomi et al.,).

### **The Quebec Ice Storm of 1998; Impact of Objective Maternal Stress on the DNA Methylation and Health Profiles of the Children**

To understand whether epigenetic differences that emerge between people that were exposed to early life stress are indeed causally related, we examined the DNA methylation profiles of children who were perinatally exposed to maternal stress as a result of living through the ice storm of 1998. King et al., have established an objective stress scale for these mothers who were followed for 15 years since the storm (Fig. 5). Differences in metabolic autoimmune and behavioral parameters were noted by King et al., A correlation analysis performed between the state of methylation of DNA in T cells of numerous CG positions and the scale of objective stress was performed and sites that showed high correlation were delineated. Several sites showed high correlation with maternal objective stress. This quasi experimental design provided first evidence that early life stress might cause DNA methylation changes in children and that these differences in DNA methylation are not limited to the brain(Cao-Lei et al., 2016; Cao-Lei et al., 2015; Cao-Lei et al., 2014).

- **Storm32** score

- **Threat**

- **Loss**

- **Scope**

- **Change**



**Figure 5.** DNA methylation markers assessing environmental and experiential history; Quebec ice storm 1998 (King et al.,)

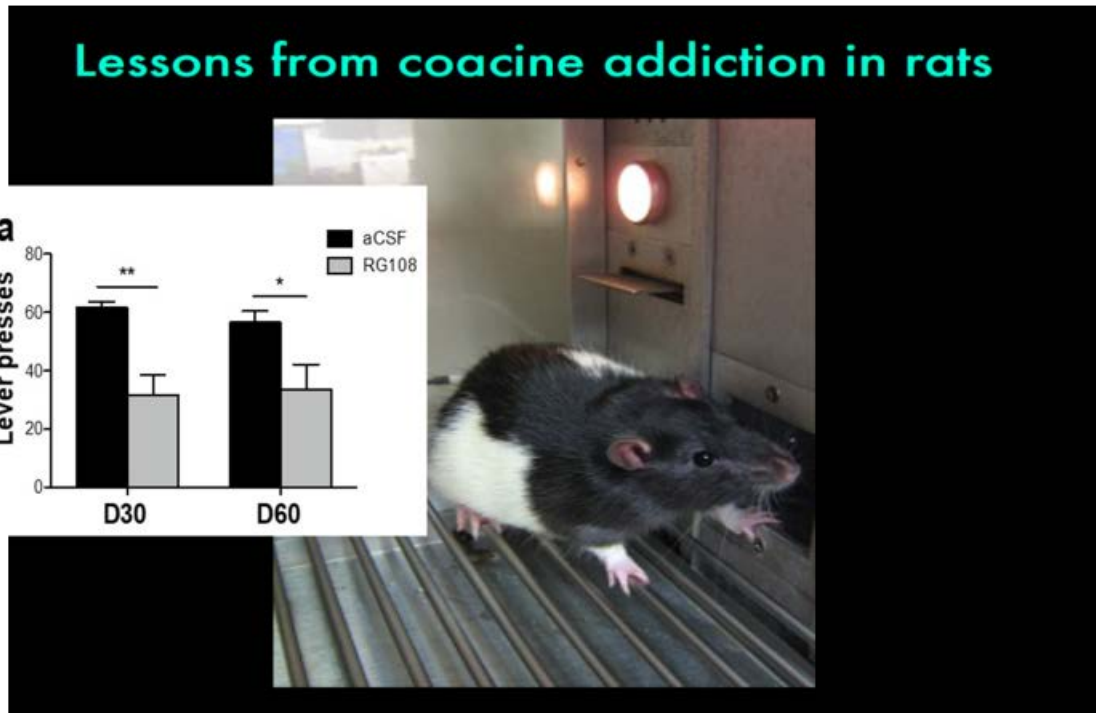
### **How can Stress Cause System Wide Changes in DNA Methylation of the Offspring?**

Social stress is perceived by the brain. Why are we noting changes in phenotype and epigenetic marking in other tissues as well? We tested the hypothesis that these changes are mediated by the glucocorticoid receptor by examining the pattern of DNA methylation at birth of placentae of mice that were deficient in one copy of the *Nr3c1* gene (the mothers had both copies intact). Differences in methylation were noted in the placentae of these mice that were sex specific. These data support the hypothesis that the glucocorticoid receptor plays an important role in mediating the emerging DNA methylation profiles in the offspring (Schmidt et al., 2019).

### **Diagnostic and Therapeutic Implications of the Epigenetic Programming by Social Exposures**

DNA methylation markers might provide additional information on behavioral exposure to help identify people at risk and provide them with additional support for effective prevention. It also raises the perspective that we will be able to treat behavioral disorders using epigenetic interventions including natural or pharmacological agents that affect epigenetic enzymes (Szyf, 2017).

For example, using a cocaine craving model developed by Yadid et al., it was shown that rats trained to self administer cocaine undergo large differences in DNA methylation in their nucleus accumbens during a month of abstinence following the initial exposure. Re-exposure to the initial cue revealed intensification of craving of the rats that abstained for one month (Figure 6). Treatment with the DNA methylation inhibitor RG108 reverses the craving state while the methyl donor SAME intensifies it. These experiments and others point to the possibility of using epigenetic treatments to reverse behavioral disorders (Massart et al., 2015; Szyf, 2017).



**Figure 6.** Lessons from cocaine addiction in rats (Yadid et al.)

## Summary

Epigenetic mechanisms provide a possible mechanism explaining the dynamic interactions between experience and the genome. They offer a different way of looking at health disease and new ways for approaching prevention and intervention.

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# Effect of Wheat Straw Chop Length in a High-Straw Dry Cow Diet on Intake, Behaviour and Health of Dairy Cows

## Effet de la longueur des particules de paille de blé dans une ration riche en paille pour vaches taries sur la consommation, le comportement et la santé des vaches laitières

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### Abstract

The transition period is a stressful time for dairy cows as changes in energy demand and supply can be difficult to adapt to. Maintaining consistent intake in the weeks leading up to calving can improve intake after calving and promote overall health of the cow. The objective of this study was to determine the effect of wheat straw chop length in a high-straw dry cow diet on intake, feed sorting, and health of dairy cows. Holstein cows (n=40) were enrolled at dry off (~45 d prior to expected calving) and assigned to 1 of 2 treatments, a high-straw (29% wheat straw on DM basis; 13.2% CP, 1.5 Mcal/kg NE<sub>L</sub>) dry cow diet with straw chopped with a: 1) 10.16cm screen (**LDD**; n=20), or 2) 2.54cm screen (**SDD**; n=20). At calving all cows were fed the same lactating TMR (16.0% CP, 1.64 Mcal/kg NE<sub>L</sub>) for 28 d. Cows on the SDD treatment had greater DMI (15.6 vs 15.0 kg/d; SE=0.16; *P*=0.02) in the dry period, while LDD cows experienced a more rapid drop (*P*<0.05) in DMI in the week prior to calving. Regardless of treatment, during the dry period, cows sorted against the long and in favor of the short particles; cows on the LDD treatment sorted to a greater extent than cows on the SDD treatment (80.2 vs 88.4 % of predicted intake; SE=2.0; *P*≤0.01). Across treatments, cows sorted in favor of the long particles (105.9±2.7% of predicted intake; *P*=0.01) and against the fine particles (91.8±1.8% of predicted intake; *P*=0.01) during wk 1 post-calving. There were no differences in rumen pH between treatments in the dry and lactating period (*P*≥0.6), although LDD cows tended to have a greater decline in rumen pH in wk 1 post-calving (*P*=0.07). Cows on the LDD had higher BHB in the wk 3 post-calving (1.3±0.11 vs 0.8±0.10 mmol/L; *P*=0.05). The results suggest that reducing the chop length of straw in dry cow diets may improve pre-calving intake, reduce feed sorting, and promote greater health across the transition period.

**Keywords:** dry cow diet, sorting, health

### Résumé

La période de transition est une période stressante pour les vaches laitières, car celles-ci peuvent éprouver des difficultés à s'adapter à la variation des besoins énergétiques et aux nouvelles sources d'approvisionnement en énergie. Le maintien de la prise alimentaire au cours des semaines précédant le vêlage peut améliorer la consommation après le vêlage et favoriser la santé générale de la vache. L'objectif de cette étude était de déterminer l'effet de la longueur des particules de

paille de blé dans une ration à teneur élevée en paille pour vaches tarées sur la consommation alimentaire, les habitudes de tri des aliments et la santé des vaches laitières. Des vaches Holstein (n=40) tarées (~45 j avant le vêlage prévu) ont reçu un de deux traitements, soit une ration à teneur élevée en paille (29 % de paille de blé sur une base MS; 13,2 % PB, 1,5 mcal/kg ÉNL) contenant de la paille hachée tamisée avec : 1) tamis de 10,16 cm (vaches « Long » [particules longues]; n=20), ou 2) tamis de 2,54 cm (vaches « Short » [particules courtes]; n=20). Au vêlage, toutes les vaches ont reçu la même RTM de lactation (16,0 % PB, 1,64 mcal/kg ÉNL) pendant 28 jours. Une CVMS supérieure a été enregistrée chez les vaches à particules courtes (15,6 contre 15,0 kg/j; écart-type=0,16; P=0,02) pendant la période de tarissement, tandis que les vaches à particules longues ont connu une baisse plus rapide (P<0,05) de la CVMS durant la semaine précédant le vêlage. Quel que soit le traitement, pendant la période de tarissement, les vaches ont préféré les particules courtes aux particules longues; les vaches à particules longues ont trié davantage que les vaches à particules courtes (80,2 vs 88,4 % de la consommation prévue; écart-type=2,0; P≤0,01). Pour l'ensemble des traitements, les vaches ont trié en faveur des particules longues (105,9±2,7 % de la consommation prévue; P=0,01) et au détriment des particules courtes (91,8±1,8 % de la consommation prévue; P=0,01) pendant la semaine 1 après le vêlage. Aucune différence de pH dans le rumen n'a été observée entre les traitements pendant les périodes de tarissement et de lactation (P≥0,6), bien que les vaches à particules longues aient connu une plus forte baisse du pH ruminal pendant la semaine 1 après le vêlage (P=0,07). Les vaches à particules longues ont présenté un taux de BHB plus élevé dans la semaine 3 après le vêlage (1,3±0,11 vs 0,8±0,10 mmol/L; P=0,05). Les résultats suggèrent qu'une réduction de la longueur des particules de paille dans l'alimentation des vaches tarées pourrait améliorer la consommation avant le vêlage, réduire le tri des aliments et favoriser une meilleure santé pendant la période de transition.

## Introduction

The transition period, defined as 3 weeks pre-calving to 3 weeks post calving (Drackley, 1999), is a challenging time for dairy cattle as it may be difficult for them to adapt to changes in energy demand and supply. Ensuring consistent feed intake across this time period reduces the risk of entering a state of negative energy balance (**NEB**) and, consequently, the risk of developing metabolic diseases. Researchers have shown that fresh, lactating cow DMI can be enhanced through a variety of nutritional means (van Saun and Sniffen, 2014). In addition to modification of the lactating cow diet, we know that the diet consumed by dry cows may be equally important in terms of stimulating intake in early lactation and promoting a smooth transition (Goldhawk et al., 2009). A common trend in transition cow management is to feed dry cows a controlled-energy diet which incorporates low nutrient dense ingredients, such as wheat straw, in order to control energy intake (Janovick and Drackley, 2010). While such diets have proved beneficial for promoting improved energy balance after calving (Janovick et al., 2011), from a cow eating behavior standpoint, one potential area of concern with such a feeding strategy is dietary selection (sorting). Although not yet studied, dry cow diets that are high in straw and low in moisture content could be hypothesized to be more prone to sorting (Leonardi et al., 2005). This may lead to cows consuming a more energy-dense diet than intended, and this sorting behavior may persist into the fresh period. There is much research to suggest that chopping forages shorter will result in not only greater DMI (Maulfair and Heinrichs, 2013), but may also help to reduce the amount of feed sorting in lactating cow diets (Kononoff et al., 2003). Thus, the primary objective of this study was to determine if pre-

calving DMI can be improved, if feed sorting can be discouraged, and if consistency in DMI can be maintained after calving by feeding dry cow diets with shorter chopped wheat straw. A secondary objective was to determine if metabolic health and post-calving performance can be improved by feeding a dry cow diet with shorter chopped straw.

## Materials and Methods

The use of cows and experimental procedures complied with the guidelines of the Canadian Council on Animal Care (2009) and were approved by the University of Guelph Animal Care Committee. Forty Holstein cows (parity =  $1.5 \pm 0.88$ ; mean  $\pm$  SD) were used in this study, which took place at the University of Guelph Livestock Research and Innovation Centre – Dairy Facility (Elora, Ontario, Canada). At approximately 45 d prior to expected calving (actual =  $42 \pm 4.2$  d), cows were dried off and enrolled in the trial. Upon dry off, cows were randomly assigned to 1 of 2 dietary treatments, a dry cow TMR that differed in the length of wheat straw: 1) straw chopped with a 2.54-cm screen (**SDD**;  $n = 20$ ) or 2) straw chopped with a 10.16-cm screen (**LDD**;  $n = 20$ ). Treatment allocation was balanced for parity and previous 305 d milk production. Upon calving, all cows were fed the same lactating cow ration formulated to meet the nutrient requirements of dairy cows producing 36 kg/d (NRC, 2001), and cows were monitored for 28 d. Feeding behavior and DMI were monitored using the automated feed bins, reticulorumen pH was measured using wireless telemetry boluses, and blood metabolites were collected to determine energy balance. Throughout the study, fresh feed samples of each diet (dry treatment diets and lactating diet) and refusal samples from each cow were collected to determine DM, particle size distribution and sorting. Particle size distribution was measured using a 4-screen Penn State Particle Separator (PSPS; Maulfair et al., 2011; Heinrichs, 2013), which separated the sample into 4 fractions based on particle size: long ( $> 19$  mm), medium ( $< 19, > 8$  mm), short ( $< 8, > 4$  mm), and fine ( $< 4$  mm). Separated samples were then oven dried at  $55^{\circ}\text{C}$  for 48 h. The sorting of each PSPS fraction was calculated (as per Leonardi and Armentano, 2003) by dividing the actual amount of feed consumed of each fraction by the predicted amount of feed consumed of that fraction and expressing it as a percentage. For each fraction, the actual amount consumed was calculated by subtracting the DM refused from the DM offered, as determined by the PSPS analyses. The predicted amount consumed for each fraction was calculated as the product of the DMI of the total diet multiplied by the DM percentage of that fraction in the fed TMR. If the sorting value equaled 100%, then no sorting of the particle fraction occurred, a value  $< 100\%$  indicated sorting against that particle size fraction, while a value  $> 100\%$  indicated sorting in favor of that particle fraction.

All statistical analyses were conducted using SAS 9.4 software (SAS Institute Inc., 2013). Due to technical failure of reticulorumen pH boluses, complete datasets were not available for all cows. Remaining analyses were conducted using a sample size of 15 cows (LDD,  $n = 7$ ; SDD,  $n = 8$ ). Significance was declared at  $P \leq 0.05$  and tendencies were reported if  $0.05 < P \leq 0.10$ . Data were first organized by status (dry or lactating) and then summarized either by day or by week (depending on sampling frequency) and analyzed using the MIXED procedure of SAS, by status, treating week or day as a repeated measure and cow as the subject of the repeated statement. The model included the fixed effects of week/day, treatment and the week/day  $\times$  treatment interaction.

## Results

Cows on the SDD had greater DMI (15.6 vs 15.0 kg/d; SE=0.16;  $P=0.02$ ) across the dry period, while LDD cows experienced a more rapid drop ( $P<0.05$ ) in DMI in the week prior to calving. Dietary treatment did not influence DMI post-calving. Cows on the SDD treatment consumed +0.4 kg/ meal ( $P=0.04$ ), tended to have 0.6 fewer meals/day ( $P=0.06$ ) and tended to consume their feed at a faster rate than cows on the LDD treatment ( $P=0.09$ ). Regardless of treatment, during the dry period, cows sorted against the long (>19 mm) and in favor of the short (<8, > 4 mm) particles, but cows on the LDD treatment sorted against the long particles to a greater extent than cows on the SDD treatment (80.2 vs 88.4 % of predicted intake; SE=2.0;  $P\leq 0.01$ ). Dietary treatment did not influence sorting post-calving; however, interestingly across treatments, cows sorted in favor of the long particles (105.9±2.7% of predicted intake;  $P=0.01$ ) and against the fine (<4 mm) particles (91.8±1.8% of predicted intake;  $P=0.01$ ) during wk 1 post-calving. There were no differences in rumen pH between treatments in the dry and lactating period ( $P\geq 0.6$ ), although LDD cows tended to have a greater decline in rumen pH in week 1 post-calving ( $P=0.07$ ). Cows on the LDD treatment had higher BHB in the third week post-calving (1.3 vs 0.8 mmol/L; SE= 0.11;  $P=0.05$ ) and, although not significant, maintained numerically higher levels of NEFA post-calving compared to cows on the SDD treatment (0.6 vs 0.5 mmol/L; SE= 0.04;  $P=0.36$ ).

## Conclusion

The results suggest that reducing the chop length of straw in dry cow diets improved intake in the dry period and minimized the drop in DMI in the week leading up to calving. Sorting against the longest particles was also minimized when cows were fed the dry diet with the shorter chopped straw. Rumen pH was more stable in the first week post-calving when cows were fed the shorter chopped straw dry diet, which is likely the result of cows on the SDD treatment having more consistent intake in the dry period and sorting less. Lastly, the dry diet with the shorter chopped straw improved metabolic health post-calving, such that SDD treatment cows had lower BHB levels 3 weeks after calving.

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## Influence of Dietary Micro-Minerals on the Intestinal Health of Broilers

### Influence des micro-minéraux sur la santé entérique des poulets à griller

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#### Abstract

The incidence of coccidiosis and necrotic enteritis (NE) in broiler chickens may increase worldwide due to mounting pressure to limit the use of sub-therapeutic antibiotics and ionophores for coccidia suppression/prevention in the diets of broilers. For this reason, we are needing to expand our knowledge on the role micro-minerals have in modulating the intestinal physiology, immunology and microbiology of broiler chickens. Zinc (Zn) is an essential micromineral required for growth, and influences intestinal development and/or regeneration during and after enteric disease. Studies have shown the impact of Zn on growth performance and the antioxidant system, immune defense and inflammation, intestinal microbial community, and intestinal permeability. It has been demonstrated that dietary Zn concentrations higher than the 40 mg/kg recommended by NRC (1994) lowers the impact of coccidiosis in broilers. Inorganic sources of Zn have been used for many years but are known to be less available for absorption than organic sources. Thus, it is reasonable to argue that when the absorptive capacity of the intestine is impaired, a more available source of Zn may be needed. Additionally, two studies were conducted by our lab to determine the effects of Zn source in broilers under coccidia and *Clostridium perfringens* challenge. In the first study, Zn proteinate had beneficial effects on the performance of chickens challenged with coccidia plus *C. perfringens* by enhancing intestinal integrity and partially attenuating the inflammatory response. In the second study, Zn proteinate lowered the expression of pro-inflammatory cytokines and modulated the ileal microbiota. Further exploration is needed to better understand the mechanisms of cellular and tissue availability from different micromineral sources during enteric challenges.

#### Résumé

L'incidence de coccidiose et d'entérite nécrotique (EN) chez les poulets à griller peut augmenter dans le monde, à cause de la pression croissante de limiter l'usage des antibiotiques sous-thérapeutiques et des ionophores pour éliminer/prévenir les coccidiens dans les régimes des poulets à griller. Pour cette raison, les microéléments peuvent être bénéfiques en modulant la physiologie intestinale, l'immunologie et la microbiologie des poulets à griller. Le zinc (Zn) est un microélément essentiel

requis pour la croissance, alors qu'il influence le développement intestinal et/ou la régénération durant et après les maladies entériques. Des études ont montré l'impact du Zn sur le rendement de croissance et sur le système antioxydant, la défense immunitaire et l'inflammation, la communauté microbienne intestinale et la perméabilité intestinale. On a démontré que les concentrations alimentaires de Zn dépassant 40 mg/kg recommandées par le NRC (1994) diminuent l'impact de la coccidiose chez les poulets à griller. Des sources inorganiques de Zn sont utilisées depuis plusieurs années, mais on sait qu'elles sont moins susceptibles d'être absorbées que les sources biologiques. Il est donc raisonnable d'avancer que lorsque la capacité d'absorption de l'intestin est compromise, une source plus disponible de Zn peut être nécessaire. De plus, notre laboratoire a réalisé deux études pour déterminer les effets des sources de Zn sur les poulets à griller testés aux coccidiens et à *Clostridium perfringens*. Dans la première étude, le protéinate de Zn a eu des effets bénéfiques sur le rendement des poulets testés aux coccidiens, plus *C. perfringens*, en améliorant l'intégrité intestinale et en atténuant partiellement l'inflammation. Dans la seconde étude, le protéinate de zinc a réduit l'expression des cytokines pro-inflammatoires et modulé le microbiote iléal. Une exploration plus approfondie est requise pour mieux comprendre les mécanismes de la disponibilité cellulaire et tissulaire de différentes sources de microéléments durant les tests de provocation entériques.

## Introduction

Macro and microminerals have long been known to exert both positive and negative effects to the gastrointestinal tract (GIT) of the animal. Chemically speaking, the form in which the cation is presented is related to a number of issues. For example, many trace minerals (e.g. Fe and Cu) can act as pro-oxidants, reduce the stability of vitamins and enzymes and promote oxidation of lipids (Cohen, 2014). Yet, finely ground  $\text{CuSO}_4$  results in increased rates of fat oxidation in feed as compared to coarsely ground  $\text{CuSO}_4$  (Miles et al., 1998). Form of the mineral will also affect its handling and storage in the milling system (sulfates, for example are highly hygroscopic and can be corrosive). Conversely, mineral disassociation within the digestive tract can also affect its reactivity with the GIT, as well as with the formation of soluble and insoluble complexes with other nutrients.

Probably the widest known example of this is that of phytin with its 12 reactive sites (6 strongly acidic, 2 weakly acidic, and 4 very weakly acidic; Angel et al., 2002). Thus, macro and micromineral cations (particularly Ca, Zn, and Cu) bind readily to phytate as pH increases above 4 forming soluble and insoluble complexes affecting the hydrolytic functions of phytase (Maenz et al., 1999; Angel et al., 2002). Further, proteins can bind directly to phytic acid through electrostatic charges or salt bridges (Graf, 1996) as well as with starch through hydrogen bond formation (Thompson, 1996). There is still a debate about the consistency of amino acid and energy released when phytase alone is supplemented to diets (Adeola and Sands, 2003), but the accessibility to phytin stored in the seed can be increased through other supplemental enzymes such as carbohydrases (Adeola and Cowieson, 2011).

Additionally, organic or chelated forms of microminerals may have higher bioavailability than inorganic sources, mainly because of their different route of absorption and lower interaction with other dietary constituents, such as phytin. Chelated minerals are metallic ions bound to a organic substances, a ligand, such as amino acids, peptides, or polysaccharides that create a stable and

soluble ion with high bioavailability (Mellor, 1964). Chelated minerals show superior absorption than inorganic minerals because usually the mineral is absorbed through the path of the organic molecule that the ion is bound which avoids its interaction with other minerals (Kratzer and Vohra, 1996). According to these authors, the mechanism in which the ligand improves the use of the mineral depends upon the capacity of the ligand to bind to the mineral, or its capacity to compete with other ligands, creating soluble complexes with the mineral.

There are several factors that influence the bioavailability of trace minerals, such as mineral concentration in the diet, source of the mineral, digestibility of the diet, particle size, interaction with other components of the diet, feed processing, strain and age of the animal, among others (Miles and Henry, 2000). The integrity of the intestine is another point that must be considered, because the absorption of minerals as well as other nutrients may be affected during enteric infections. Additionally, the distribution of minerals within the body of the animal may change during infection (Turk and Stephens, 1966, 1967; Southern and Baker, 1983; Turk, 1986; Richards and Augustine, 1988; Bortoluzzi et al., 2019b). These authors reported that the plasma concentration of Zn decreases but increases in the liver during coccidial and bacterial challenge in broiler chickens. Nevertheless, the effect of Zn on immunity is well known (Chevalier et al., 1996). The complex Zn-methionine may also be more available than inorganic sources of Zn and be absorbed without structural changes, which alters the balance of this mineral in the animal's organism. Regardless of the mechanism, when Zn-methionine is added to diets of broilers or passed through the yolk, there is an improvement in the resistance against some disease (Kidd and Kerr, 1996). It has been reported that Zn and Mn chelated to methionine improved the immune function of turkeys (Ferket and Qureshi, 1992). The antimicrobial effect of Cu has also been shown, mainly by its effects in reducing the concentration of *Escherichia coli* in the intestine of broilers when added in high concentration to the diet (Pang et al., 2009; Klasing and Nazipipour, 2010). Therefore, the objective of this review is to provide insights into the role of microminerals in mitigating the negative impact of enteric diseases in broilers.

## **Mineral Interactions Within the GIT**

The mineral cation-phytin complex that forms within the GIT, theoretically explains much of the differences in efficacy that it is observed between phytase sources with differing pH profiles, with much of the improvement with today's generation of phytases occurring because the profiles favor hydrolysis at more acidic pH where the cation-phytin complexes are more soluble (Maenz et al., 1999; Tamim and Angel, 2003; Tamim et al., 2004; Pang and Applegate, 2006). However, the consistencies of results observed in vitro with mineral cations are not always translated into predictable and quantifiable results across ages and mineral sources within and between research labs. For example, across 5 studies with Cu in broiler chicks (Banks et al., 2004a,b; Pang and Applegate, 2007; Pang et al., 2009), Cu was not effective in reducing apparent ileal phosphorus (P) or phytate-P (PP) hydrolysis when included up to 188 mg/kg of diet. However, when the dietary Cu concentration was increased to 250 mg/kg, it reduced apparent ileal P digestibility by 0.03 to 0.06 (as a % of the diet). There appears to be differences between Cu sources, but the results across experiments are not consistent. Similarly, a threshold of dietary concentrations needed to affect *in vivo* P digestibility or PP hydrolysis may exist. For example, results from a turkey poult study where up to 161 mg/kg of dietary Zn was fed showed no differences in apparent ileal P

digestibility or PP hydrolysis (Applegate et al., unpublished results). Thus, the concentration of dietary Cu and Zn needed to have a consistent negative impact on P digestibility or PP hydrolysis may be above concentrations used in practical poultry diets.

Calcium, while on one hand forms weaker complexes with individual phytate molecules, may bring more to bear due to sheer quantities in the diet (Tamim and Angel, 2003; Tamim et al., 2004). These authors demonstrated the effect of Ca on PP digestibility by adding EDTA, a stronger chelator of Ca (vs. phytate), to a broiler diet containing 0.7% Ca. When this diet was fed to broilers PP digestibility was similar to that seen when a diet devoid of added Ca (0.21% Ca) was fed in a short term experiment. Thus, in practical broiler diets, presence of Ca inhibits 0.03 to 0.13% of dietary phytate P hydrolysis (Applegate et al., 2003; Tamim and Angel, 2003; Tamim et al., 2004).

While we know the disassociated cation from some macro- and micro-mineral sources is highly reactive with molecules such as phytate, the poultry industry has included prophylactic concentrations of Cu and Zn in the diet well beyond what has been indicated to prevent deficiency symptoms. Historically, high concentrations of Cu were originally ‘touted’ as having benefits in prevention of crop mycosis. Indeed, field studies indicate it does have some merit, but reproducibility of induced crop mycosis in experimental conditions has had less than favorable results (Underwood et al., 1956). In fact, addition of up to 250 mg Cu/kg diet results in increased erosion to the lining of the gizzard (Fisher et al., 1973; Poupoulis and Jensen, 1976) and results in an “inhibition of normal fermentation” in the cecae of the chick (Jensen and Maurice, 1978). This observation has been confirmed in *in vitro* anaerobic digestion. In particular, volatile fatty acid production can be inhibited considerably due to reductions in microbial activity (Yenigün et al., 1996).

## **Zinc**

Zinc is an essential micromineral required for growth, and influences intestinal development and/or regeneration during and after enteric diseases (MacDonald, 2000). The indispensability of Zn in the diets of animals has been recognized for years (Salim et al., 2008). Zinc is a hydrophilic ion and cannot cross cellular membranes by simple diffusion, which requires specialized mechanisms for its cellular uptake. Integral membrane transport proteins are used to move Zn across the lipid bilayer of the plasma membrane (Tako et al., 2005). Over the years, inorganic sources of Zn have been used as oxides and sulfates to supplement the diets of broiler chickens above the NRC (1994) recommended concentrations (Lesson, 2005).

When inorganic trace minerals are fed and reach the upper parts of the GIT they tend to dissociate due to the low pH and interact with other minerals or dietary compounds (Mwangi et al., 2017), decreasing their bioavailability. Thus, it is reasonable to argue that when the absorptive capacity of the intestine is impaired due to an enteric infection, such as coccidiosis and NE, a more available source of Zn may be needed. The use of organic Zn to supplement broiler diets is becoming a more common practice when looking to enhance mineral uptake, improved growth performance, and reduce mineral excretion (Yan and Waldroup, 2006; Burrell et al., 2004; Nollet et al., 2007; Mwangi et al., 2017). Zinc nutrition has become an active area of research, mainly in broilers. Adequate Zn intake and absorption is essential for many metabolic and biological functions, including growth, reproduction, meat quality, and immune response against pathogens challenge (Salim et al., 2008).

Higher concentrations of Zn are also rationalized based on literature wherein Zn metabolism shifts with coccidial and bacterial challenges. Specifically, plasma Zn greatly decreases (Turk and Stephens, 1966, 1967; Southern and Baker, 1983; Turk, 1986; Richards and Augustine, 1988) while liver Zn greatly increases as it is bound through up-regulation of metallothionein (and presumably intra-cellular Zn-transporters (ZnT) and Zn-regulated transporter proteins (ZIP)) during an acute phase response to these challenges (Richards and Augustine, 1988). Indeed, severity of growth depression has been observed to be lessened when supplemental Zn was increased to 85-90 mg Zn/kg diet (Turk and Stephens, 1966, 1967; Bafundo et al., 1984). However, it should be noted that while we would presume a malabsorption during an intestinal insult (such as that of coccidiosis), a more accurate description of Zn metabolism is that of a re-distribution of Zn pools within the body as well as intra-cellularly.

Recent work has further elucidated intra-cellular roles of Zn during coccidial challenge. Interestingly, during a mild coccidial vaccine challenge, phagocytic capacity increases while intracellular free Zn is reduced in cecal tonsils (Troche, 2012). Intestinal mucosal tissues increased their expression of ZIP transporters (specifically ZIP 9 and 13) to prepare for future need to transport Zn into the cytoplasm from intra- and extra-cellular storage. In this work, level of dietary Zn and source had no influence on these processes. However, subtle differences were observed in parallel work, wherein intestinal tissues from birds challenged with a mild coccidial vaccine were exposed to a secretory stimulant (carbachol), birds fed a moderately deficient Zn diet dramatically increased mucosal secretion of Cl<sup>-</sup> (Troche, 2012). Feeding of ZnSO<sub>4</sub> alone (90 mg Zn/kg diet) alone was unable to mitigate this hyper-chloride secretion/ anaphylactic response, whereas a blend of ZnSO<sub>4</sub> and a Zn amino acid complex was able to bring Cl<sup>-</sup> secretion back to levels of unchallenged birds. In a recent publication from our lab (Bortoluzzi et al., 2019b), it was observed that *E. maxima* plus *C. perfringens* challenge upregulated the expression of ZIP 13 and downregulated ZnT 7 in the jejunum of broilers, but in the cecal tonsils the expression of all Zn transporters was downregulated; yet, Zn supplementation (90 mg/kg), regardless of the source, downregulated the expression of ZnT 5. Thus the form of delivery of Zn to tissues along the digestive tract may play a functional role in intra-cellular responses to pathogens.

Zinc is paramount for adequate functioning of heterophils, mononuclear phagocytes and T lymphocytes (Kidd et al., 1996). Furthermore, studies have shown the impact of Zn on growth performance and the antioxidant system (Mwangi et al., 2017); immune defense and inflammation (Prasad et al., 2011; Li et al., 2015; Bortoluzzi et al., 2019ab), intestinal permeability (Zhang and Guo, 2009; Bortoluzzi et al., 2019a; Bortoluzzi et al., 2019b), and intestinal microbiota (Bortoluzzi et al., 2019b). *Eimeria acervulina* infection has been shown to reduce the effect of Zn toxicity in chickens when added to diets in high concentrations (2,000 and 4,000 mg/kg), most likely due to the impaired absorption rate (Southern and Baker, 1983). More recently, (Troche, 2012) showed that the Zn concentration required for maximum body weight gain in chickens went from 45 mg/kg in uninfected chickens to 75 mg/kg in coccidiosis infected birds. Therefore, enteric infections impair the proper absorption of Zn which may become deficient to promote adequate growth and functioning of important systems.

Additionally, organic Zn induced higher expression of A20, an anti-inflammatory regulator, downregulated the expression of inflammatory inducers, including NF-kB p65 (Prasad et al., 2011; Li et al., 2015), and promoted MUC2 and IgA production, when compared to its inorganic

counterpart (Prasad et al., 2011). Epigenetic mechanisms alter gene expression without changes in DNA sequence and can explain the effects of Zn on the cell. The higher expression of A20 promoted by organic Zn is most likely due to an epigenetic effect by lowering DNA methylation (Li et al., 2015). In two recent publications from our lab (Bortoluzzi et al., 2019a; Bortoluzzi et al., 2019b) it has been demonstrated that organic Zn had significant impact in lessening the inflammatory response in the intestine by down-regulating the expression of pro-inflammatory cytokines, such as IL-8 and  $\text{ING-}\gamma$ , TLR-2 and iNOS. Additionally, it has been reported that organic Zn reduced the intestinal permeability caused by *Clostridium perfringens* challenge on top of coccidiosis (Bortoluzzi et al., 2019a), or regardless of the source, Zn had no impact on the intestinal permeability (Bortoluzzi et al., 2019b).

Changes in intestinal permeability may be influenced by modulation (down or up-regulation) and/or functionality of TJ proteins, in which bacterial derived proteases may cause its degradation (Awad et al., 2017) by a broad range of mechanisms. However, it has been demonstrated that *C. perfringens* enterotoxins are able to attach to the cell surface by binding to the TJ proteins, especially to the claudin family proteins (Eichner et al., 2017). With the objective of understanding the molecular basis of the reduction of the intestinal permeability promoted by Zn supplementation, (Zhang and Guo, 2009) evaluated the gene expression of TJ proteins in weaning piglets as well as protein expression by Western blots. They observed that Zn enhanced intestinal permeability, by upregulating the expression of occludin and zonula occludens- 1 at the mRNA and protein levels. In another study, Zhang et al. (2012) observed that supplemental Zn upregulated the expression of occludin and claudin-1 in the ileum of chickens challenged with *Salmonella* Typhimurium. As mentioned, it was observed that organic Zn reduced the intestinal permeability without effect on the expression of TJ genes (Bortoluzzi et al., 2019a); yet, the challenge with *E. maxima* plus *C. perfringens* upregulated the expression of claudin-1, and downregulated occludin and zonula occludens expression, without effect of Zn on the expression of these genes nor on the intestinal permeability (Bortoluzzi et al., 2019b).

Despite to the fact that Zn is widely used in animal nutrition in high concentrations with the objective of promoting growth, there is a lack of studies available on the effects of Zn on the intestinal bacterial community (Starke et al., 2014). These authors evaluated the effect of high dietary Zn (2,425 mg/kg) in the diet of weaned piglets on the intestinal microbiota and their metabolic activity and observed that the diet had a large impact on three species of *Lactobacillus* mainly. *Lactobacillus amylovorus* was the most impacted species, wherein its decrease coincided with lower lactic acid concentrations due to the high dietary Zn. Yet, Bortoluzzi et al. (2019b) reported that organic Zn supplementation reduced the amount of *Lactobacillus* in the ileal digesta, and *Coprobacillus* in the cecal content. Furthermore, it seems that high dietary Zn concentrations have no effect (Broom et al., 2006) or increase the number of enterobacteria in the gut (Højberg et al., 2005), which may suggest that these bacteria possess a mechanism to counteract high Zn concentrations (Starke et al., 2014). Yet, bacterial metabolites in the small and large intestines were lower in the high dietary Zn group, mainly because of a decrease in propionate concentration, and partially due to lower n-butyrate concentrations. On the other hand, Zn supplementation (120 mg/kg) restored the cecal microbial community of *Salmonella* Typhimurium infected chickens by increasing the number of total bacteria and *Lactobacillus*, and reducing *Salmonella* colonization (Shao et al., 2014). Therefore, it seems that the effect of Zn supplementation relies on the concentration and form of delivery that it is used in the diet and the presence, absence, and/or severity of a challenge.

## Copper

Several variables may undergird our knowledge of how source and/or form of Cu may affect intestinal microbiota. For example, Pang et al. (2009) reported that while classical plate enumeration of *E. coli* was linearly reduced from media inoculated with ileal digesta from birds fed increasing dietary CuSO<sub>4</sub> (to 250 mg/kg), but was unaffected by tri-basic copper chloride (TBCC) supplementation up to 250 mg/kg. Conversely Klasing and Nazipipour (2010) observed increased bacteriostatic activity against *E. coli* spiked into ileal content when 150 mg Cu/kg diet from TBCC but not from CuSO<sub>4</sub> was fed. Interestingly, their report related differences observed between Cu sources to that of Cu solubility and extractability from its chemical source. Notably, when CuSO<sub>4</sub> was fed, duodenal luminal soluble Cu and epithelial metallothionein was increased vs that from TBCC fed birds. Along the entire small intestine, TBCC resulted in more ethylenedis-hydroxyphenylglycine (EHPG; a strong complexing agent) extractable Cu, thereby suggesting improved bio-availability throughout the length of the GIT. The authors related this difference in Cu extractability then to the bacteriostatic activity observed from feeding the TBCC.

Both the vertical (proximal to distal) and horizontal (luminal to epithelial-associated) distribution of microbiota may then be dependent upon micromineral source and where the mineral is extracted and subsequently absorbed in the luminal/mucosal interface. To this end, Pang et al. (2009) noted no change in number of predominate bacterial populations of either the ileal digesta or intestinal mucosa utilizing a general bacteria-specific PCR primer targeting conserved regions of the V3 region of 16S rRNA. Further, source of Cu (TBCC or CuSO<sub>4</sub>) had no impact on the diversity of bacteria in the intestinal digesta. However, feeding of 187.5 mg/kg Cu from TBCC increased the similarity of bacterial microbiota between birds compared to either control or birds fed 187.5 mg/kg Cu from CuSO<sub>4</sub>. As our technical ability to determine microbiota shifts and what that means from a host-microbial relationship improves, our poultry science community needs to further elucidate how chemical, chelate, and organic forms of Cu effect this interface.

While the exact effects of ionic Cu has on the intestinal microbiota are unresolved, the poultry industry continues to utilize prophylactic concentrations of dietary Cu for its ability to improve feed conversion. One of the first reports of Cu supplementation having a growth promoting (rather a feed efficiency improvement) was those of Mehring et al. (1960). However, in studies with penicillin and streptomycin, Weeks and Sullivan (1972) noted no additional benefit when used in combination for multiple turkey experiments. Despite the positive effects of prophylactic dosages of Cu, several confounding factors exist which can cause a negative response to these high dietary concentrations. Variables such as: age, length of time on treatment, overall health and disease level, as well as the concentrations of other minerals in the diet can affect the animal's response to Cu (Kornegay, 1983).

## Manganese

A specific biochemical role for manganese in intermediary energy metabolism was confirmed when pyruvate carboxylase was discovered to be a manganese metalloprotein (Scrutton et al., 1966, 1972). Manganese is required for normal lipid and carbohydrate metabolism through the activity of pyruvate carboxylase. Defects in lipid and carbohydrate metabolism have been reported in manganese-deprived rats and guinea pigs and a diet low in manganese can reduce fat deposition

in pigs (Plumlee et al., 1956). A second function of Mn has been identified as member of superoxide dismutase (SOD) enzyme. This enzyme is needed for added protection against oxidative stress associated with inflammatory responses to some infections. Manganese deprivation lowers MnSOD activity and increases the peroxidative damage caused by high dietary levels of polyunsaturated fatty acids (PUFA).

Manganese is also needed for the synthesis of mucopolysaccharides through its activation of glycosyltransferase. Impaired glycosyltransferase activity reduces the synthesis of glycosaminoglycan and oligosaccharide side chains in animals deprived of manganese (Leach and Harris, 1997). Manganese-deprived chicks have less proteoglycan in the cartilage of the tibial growth plate than Mn replete chicks and the carbohydrate composition of monomers is changed (Liu et al., 1994). In laying hens, subnormal egg production and poor shell formation may result from impaired mucopolysaccharide synthesis (Hill and Mathers, 1968). In recent work by Ibrahim et al. (2016), it was reported that Mn reduced gastric ulceration stimulated by intra-gastric injection of acidified ethanol in rats. Also, the authors showed that Mn increased mucous thickness of the stomach and increased the activity of SOD. However, there is no information available on the role of Mn on the production of mucus in the intestine which is considered an important component of the innate immune system of animals.

Manganese has also been described as a trace mineral associated with better immunity, or to functions that support immunity (Kidd, 2004). However, there is a lack of studies relating Mn supplementation and its different presentation forms on the immune response against coccidiosis and necrotic enteritis. Recently, Burin Junior et al. (2019) have shown that birds fed organic Mn had a more efficient response against a *Salmonella* Enteritidis vaccine when compared to birds fed its inorganic counterpart. The absence of effect on the growth performance related to Mn (Burin Junior et al., 2019) may be attributable to the fact that the basal diet had enough Mn to support growth, and/or the immune stimulus was not strong enough to elucidate a deficiency of Mn. Therefore, it is necessary to understand the dynamics of use of Mn when submitting the birds to a stronger challenge, such as the exposure to *Eimeria* and *C. perfringens*.

## Conclusion

It is known that trace minerals have an important role in preventing the losses associated with intestinal infection in broiler chickens, and the use of higher concentrations may be considered an important strategy to help the animal to cope with the disease. However, information is still missing regarding the effects of dietary microminerals on microbial-cross talk and its interaction with the host, and modulation of pathogenicity factors by these minerals. Therefore, further exploration is needed to better understand the mechanisms of cellular and tissue availability from different micromineral sources and its effects during enteric challenges.

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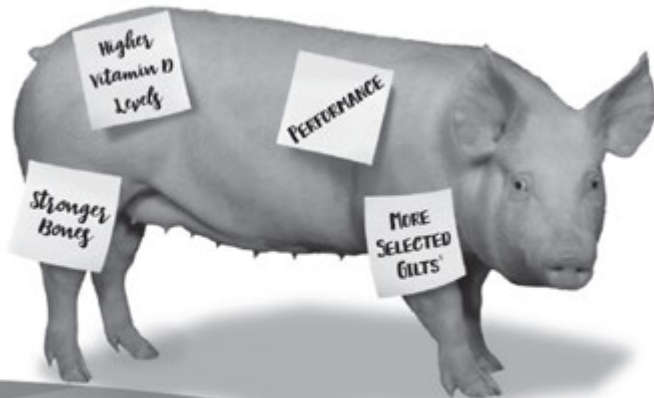
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## **Amino Acid Nutrition: Long-term Implications for Sows and Offspring**

### **Nutrition aux aminoacides : implications à long terme pour les truies et pour leur progéniture**

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#### **Abstract**

Amino acid requirements and amino acid utilization change considerably throughout the course of gestation. The empirical and factorial estimates of amino acid requirements for gestating sows are currently calculated solely using the retention of each amino acid in maternal and pregnancy-associated protein pools. These estimates do not take into account the ‘non-protein’ roles of amino acids, which can expend a significant portion of amino acid supply. Furthermore, most amino acid requirement studies for gestating sows are outdated and therefore, do not account for the metabolism of modern sows, nor the fact that sows now produce larger litters, more piglets per sow per year, and more milk during the lactation period. First-parity sows are most vulnerable to dietary deficiencies due to relatively low feed intake and high nutrient demands for continued maternal growth and growth of the products of conception. In this unique dichotomy of fetal versus maternal growth, these sows may be less able to compensate for dietary deficiencies with her own body reserves. Alternatively, the first-parity sow may sacrifice maternal reserves and thus negatively impact her own growth and development. Both of these scenarios could have long-term implications for the sow’s future productivity and longevity. The supply of dietary nutrients and energy to the gestating sow can also impact the developing fetus, which can affect piglet quality at birth, future growth performance, and longevity of female offspring destined to become replacement breeding stock. Therefore, gestational feeding programs must consider both maternal and offspring outcomes.

#### **Résumé**

Les exigences en aminoacides et l’utilisation des aminoacides changent considérablement au cours de la gestation. Les estimations empiriques et factorielles des exigences en aminoacides pour les truies en gestation sont présentement calculées en utilisant seulement la rétention de chaque aminoacide dans les bassins protéiques maternels et associés à la grossesse. Ces estimations ne tiennent pas compte des rôles non protéiques des aminoacides, qui peuvent augmenter une portion significative de l’apport d’acides aminés. En outre, la plupart des études sur les exigences en aminoacides chez les truies en gestation sont désuètes, ne prenant pas en considération le

métabolisme des truies modernes, ni le fait que les truies ont aujourd'hui de plus grandes portées, plus de porcelets par truie par année, et plus de lait durant la période de lactation. Les truies primipares sont les plus vulnérables aux déficiences alimentaires, en raison d'une prise d'aliments relativement faible et des demandes élevées en nutriments pour la croissance continue de la mère et la croissance des produits de la conception. Dans cette dichotomie unique entre la croissance du fœtus et celle de la mère, ces truies peuvent être moins capables de combler les déficiences alimentaires avec leurs propres réserves corporelles. D'un autre côté, la truie primipare peut sacrifier ses réserves maternelles et, ainsi, nuire à sa propre croissance et à son propre développement. Ces deux scénarios pourraient avoir des implications à long terme sur la productivité et la longévité futures de la truie. L'apport de nutriments alimentaires et d'énergie à la truie en gestation peut aussi toucher le fœtus en développement, ce qui peut affecter la qualité du porcelet à la naissance, le rendement de croissance futur et la longévité de la progéniture femelle destinée à remplacer les animaux reproducteurs. Par conséquent, les programmes d'alimentation gestationnelle doivent tenir compte des résultats pour les mères autant que pour leur progéniture

## **Introduction**

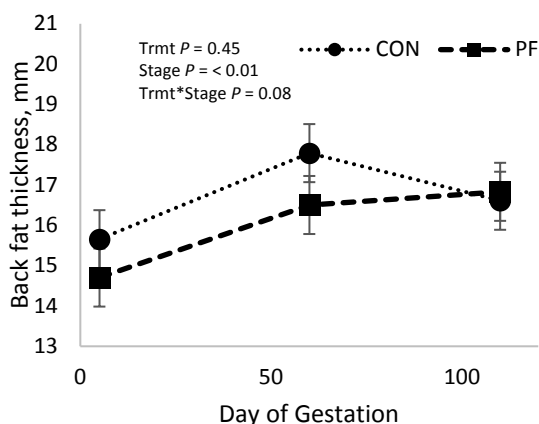
Nutrient and energy requirements for sows do not remain static throughout the various stages of reproduction. For instance, the estimated lysine requirements of a first parity sow increase by 200% between day 0 and 114 of gestation, while the estimated energy requirements increase by 45% within the same timeframe (NRC, 2012, Buis 2016). This increase in nutrient and energy requirements is largely driven by the exponential fetal growth that occurs within the last trimester of gestation. It is, however, also important to consider the unique and dynamic nutrient and energy requirements during the earlier stages of gestation as well. For example, sows must be provided with sufficient energy and nutrients to grow maternal tissues (immature sows) and to regenerate maternal protein and energy stores lost during the previous lactation (multi-parity); both processes occur largely in the first trimester before priority shifts to the growth of pregnancy-associated tissues (NRC, 2012).

The order of priority for the gestating sow is to first meet her energy requirements for maintenance (e.g., body temperature regulation), then growth of conceptus, and finally, maternal protein gain. Any additional energy intake is directed toward maternal fat deposition and, in instances of insufficient energy intake, the sow will mobilize her own tissue reserves to meet the requirements for maintenance and conceptus growth (NRC, 2012). Expansion of the pregnancy-associated (i.e. fetus, uterus, placenta and associated fluids, and udder) and maternal protein pools, constitute the majority of amino acid needs during gestation. Each of these tissues have a unique amino acid profile and the growth of each occurs at vastly different rates and at different times during gestation. For example, the placenta and fluids grow rapidly early in the second trimester but remain relatively constant in size throughout the remainder of gestation. Conversely, the fetal and udder tissues experience rapid and sustained growth during the final trimester (NRC, 2012; Trottier et al., 2015). The sow will sacrifice her own body amino acid (protein) stores to maintain pregnancy-associated growth when the diet is deficient in amino acid(s). Thus, we use visual assessments of sow body condition throughout gestation as an indicator that energy and nutrient

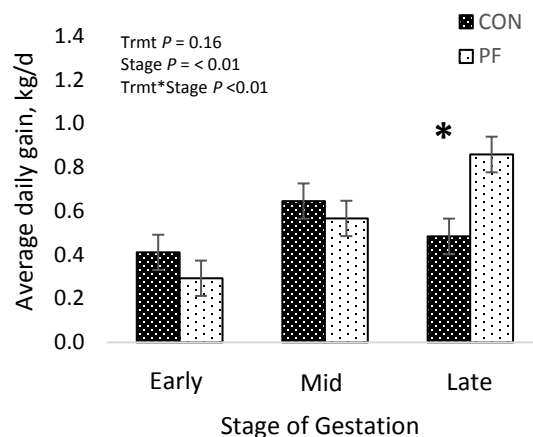
requirements were met appropriately and that partitioning of energy and nutrients between the sow and the products of conception was suitable.

## Gestation Feeding Programs

Both parity and nutritional regime (i.e. amino acid and energy supply) during gestation can influence the partitioning of nutrients within the sow herself and the division of nutrients between the maternal and fetal pools, which can have long-term implications for both the sow and her offspring. For example, we have shown that altering the SID Lys (%): (net) Energy ratio to precisely meet estimated requirements of first parity sows on each day of gestation (approximate range: 1.4 to 2.8 between day 5 and 110 of gestation) resulted in an altered back fat thickness profile throughout gestation (Figure 1) and greater body weight gain in late gestation (Figure 2) versus feeding a constant SID Lys:Energy (2.2) ratio throughout gestation (preliminary data; Stewart et al., *unpublished*). In other words, the precision feeding program supported a continuous increase in both back fat thickness and body weight gain, even when nutrient demands to support products of conception were the greatest (i.e. in late gestation). Furthermore, energy, and potentially amino acids, were limiting in the control sows by late gestation as they sacrificed both back fat and body weight gain in order to support litter growth. Despite this, the total amount of body weight gain was not different between the groups and the number of pigs born alive was not impacted, but sows fed using the precision program had fewer stillbirths and a lighter litter birth weight. The long-term consequences for both the sow and her offspring have yet to be elucidated, but are currently under investigation in our laboratory.



**Figure 1.** Back fat thickness (mm) between day 5 and 110 of gestation for first parity sows fed either a control (CON; 0.56% SID Lys and 2518 kcal/kg NE) or precision feeding (PF; unique daily blend of two diets to meet estimated daily energy and Lys requirements) regimen.



**Figure 2.** Average daily gain (kg/d) between day 5 and 110 of gestation for first parity sows fed either a control (CON; 0.56% SID Lys and 2518 kcal/kg NE) or precision feeding (PF; unique daily blend of two diets to meet estimated daily energy and Lys requirements) regimen.

In our other work, we have also shown that nutrient partitioning and offspring physiology at birth can be impacted by gestating sow feeding regimen. For example, feeding energy 15% above estimated requirements, while meeting or exceeding estimated amino acid requirements, resulted in increased maternal protein deposition and back fat thickness for 1<sup>st</sup> and 2<sup>nd</sup> parity sows versus those fed energy 15% below estimated requirements, with no adverse effects on pregnancy-associated (i.e. the sum of fetus, mammary gland, uterus, and placenta and fluids; NRC, 2012) protein deposition (Miller et al., 2016, 2017) or piglet energy reserves (i.e. body fat or tissue glycogen; Miller et al., *unpublished*). When energy was fed 15% below estimated requirements, however, piglets at birth had increased plasma IGF-1 concentrations (Miller et al., *unpublished*).

It is clear that the sow is able to buffer dietary shortcomings with her own body reserves of protein and energy, either by slowing her own growth or by catabolizing maternal tissues. It appears that the sow can do this very well, at least for a short time, and therefore, the effects of under- or over-supplying nutrients are not always easy to discern in the short-term. This is particularly true in commercial production where infrequent visual assessment is the sole measurement of body condition. Therefore, it is important to meet energy and amino acid requirements “right now” but also to consider that how we feed the sow at this moment can also influence future phases of the reproductive cycle for both the sow and her offspring. Parity-segregated, phase-feeding or precision feeding approaches appear to be the most promising strategies to meet the changing energy and amino acid requirements of gestating sows.

## **Methionine supply and fetal programming**

In order to precisely match nutrient requirements with nutrient supply (i.e. in a precision feeding program), the requirements for each nutrient must be accurately quantified. The empirical and factorial estimates of amino acid requirements for gestating sows, however, are currently calculated solely using the retention of each amino acid in maternal and each of the pregnancy-associated protein pools. These estimates do not take into account the ‘non-protein’ roles of amino acids, which can use up to 50% of amino acid supply in neonatal pigs (McBreairey et al., 2013).

Methionine is an essential amino acid that is required for protein accretion and cysteine and taurine synthesis but is also the primary methyl donor (Brosnan et al., 2007). Methionine metabolism can be divided into three major pathways: transmethylation (**TM**: methionine to homocysteine), remethylation (**RM**: homocysteine to methionine), and transsulfuration (**TS**: homocysteine to cysteine). Methyl groups supplied by methionine are important for over 50 TM reactions in the body. Each reaction is catalyzed by a specific methyltransferase, although phosphatidylcholine (**PC**; a component of cell membranes) and creatine (energy storage molecule; also requires arginine) synthesis and methionine catabolism (also requires serine and glycine) account for the bulk of methyl utilization (Stead et al., 2006; Brosnan et al., 2007). The RM of homocysteine to methionine can occur through two different pathways that use labile methyl groups or betaine

(choline). The TS of homocysteine to cysteine occurs across two irreversible reactions (Brosnan et al., 2007).

Even though methionine has been well established as an important effector of fetal programming, the most recent methionine requirements studies for gestating sows were conducted nearly 50 years ago (Holden et al., 1971; NRC, 2012); these studies do not account for the metabolism of modern sows, nor the fact that our current sows produce larger litters, more piglets weaned per sow per year, and more milk during the lactation period (NRC, 2012; Galiot et al., 2018). It is well known that the neonatal piglet has particularly high demands for methionine and methyl groups to maintain basic functions and to rapidly expand protein and non-protein (e.g., creatine, PC; Brosnan et al., 2009) pools. In suckling piglets, half of dietary methionine is used for protein synthesis while the other half is accounted for equally in creatine and PC (McBreairty et al., 2013). Creatine synthesis alone in neonatal pigs requires fourfold greater TM flux versus the entire TM flux in adult humans (Hoffer, 2002; Brosnan et al., 2009). In the context of gestation, up to 18 piglets may be developing in utero (Galiot et al., 2018), and the total methyl demand for creatine synthesis alone must be substantial. When protein retention is used as the main outcome to determine methionine requirements, methyl groups may still be limiting for TM and RM reactions, especially in late gestation, when fetal piglet growth is exponential (NRC, 2012). It is intriguing then, to think about how the sow must accommodate the considerable protein and non-protein methionine needs for her developing litter, particularly in late gestation. Does the sow alter her own methionine and methyl metabolism to meet the needs of her litter? Is there a mechanism by which the placenta is able to sequester methionine and methyl groups for preferential utilization by fetal tissues? And if so, what are the (long-term) consequences for the sow and her offspring if dietary methionine supply is inadequate?

From other animal models we know that in instances of insufficient (dietary) methyl supply, the partitioning of methionine for protein synthesis and methylation reactions is impacted. For example, when piglets (15-18 days of age) received guanidinoacetate to elicit obligatory creatine synthesis, methyl incorporation in to both PC and protein were reduced (McBreairty et al., 2013); the latter suggests that methionine was diverted away from protein synthesis. Furthermore, when fed diets deficient in both methionine and methyl precursors (i.e. folate, choline, and betaine), piglets (4-8 days of age) had reduced whole-body, muscle, and jejunal protein synthesis versus those fed diets deficient in methionine but with adequate methyl precursors (Robinson et al., 2016). Also, when sulfur amino acid-deficient diets were fed to piglets, epithelial cell proliferation in the jejunum was reduced and intestinal oxidative stress was increased (Bauchart-Thevret et al., 2009). Moreover, DNA methylation patterns can be influenced by dietary methyl supply during prenatal development (Waterland et al., 2006). Though promoter-specific, heritable DNA methylation consumes only 1% of methyl groups (McBreairty et al., 2013), it can have significant implications for subsequent metabolism and lifetime productivity (Liu et al., 2011; Myrie et al., 2012), both for the animal itself, but also for subsequent generations. The latter is noteworthy when we consider the selection of future breeding stock (males and females).

All of the research outlined thus far was conducted on piglets after birth – but what about when gestating sows are fed diets with unbalanced methionine supply? The effects on the offspring, of course, hinge on the mother's ability to compensate for diet inadequacies. Feeding unbalanced methionine during gestation has been shown to alter the expression of specific genes involved in lipid and carbohydrate homeostasis of rat offspring after weaning (Lillicrop et al., 2005) and, in ewes, dietary supplementation of methionine in late gestation improved offspring insulin sensitivity at least until the 3<sup>rd</sup> week of life (Sulaiman et al., 2017). Clearly, methionine supply during gestation can have lasting effects on offspring metabolism and potentially lifetime productivity, but similar studies have not been conducted in swine.

Dietary methionine supply during gestation may also influence the sow as sulfur amino acids are important for keratinisation in the hoof and claw (van Riet et al., 2013) and for glutathione synthesis (i.e. the primary intracellular antioxidant; Bauchart-Thevret et al., 2009). Therefore, in times of (sustained) methionine undersupply, sows may be more prone to lameness and oxidative stress, as well as, experience altered protein turnover and partitioning of methyl groups among methylation reactions themselves (McNeil et al., 2008). First-parity sows are most vulnerable to dietary deficiencies due to relatively low feed intake and high nutrient demands for continued maternal growth and growth of the products of conception (NRC, 2012). In this unique dichotomy of fetal versus maternal growth, these sows may be less able to compensate for dietary deficiencies with their own body reserves. Alternatively, the first-parity sow may sacrifice maternal reserves and thus negatively impact her own growth and development; improper nutrition and perturbed nutrient partitioning in the sow has a cumulative effect on sows across reproductive cycles (Trottier et al., 2015), which could have long-term implications for the sow's future productivity and longevity.

## **Conclusion**

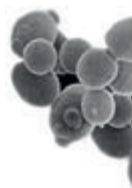
We know that the nutrient and energy requirements of sows are dynamic throughout the gestation period, but in order to precisely meet these requirements, we first must accurately quantify or predict how much of each nutrient the sow requires to support both herself and her offspring at each stage (day) of gestation. In sows however, most empirical amino acid requirement studies (with the exception of lysine) are dated, and our factorial estimates do not take into account the non-protein roles of amino acids. Methionine, in particular, fulfills many roles within the body as it is the primary methyl donor, in addition to its use for protein synthesis. Therefore, the balance of methionine partitioning between protein synthesis and TM reactions, should be considered when estimating methionine (sulfur amino acid) requirements. The consequences of not supplying sufficient methionine to sows during gestation are twofold: 1. The offspring may experience perturbed methyl metabolism after birth, which could hinder productivity and be passed to future generations (in the context of replacement breeding stock) and 2. The sow may sacrifice her own growth and development, maternal tissue stores, or alter her own methyl metabolism to meet the demands of her developing litter, which could negatively impact her future reproduction and longevity within the herd. It is clear that there is still a vast amount of research required in these areas so that we can adequately support precision feeding programs for reproductive sows.

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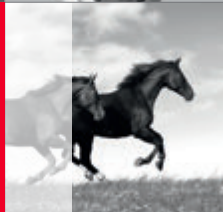
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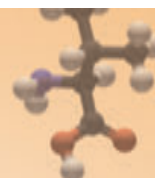
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## Effects of Diet on the Gastrointestinal Microbial Ecosystem and Gut Gene Expression in Young Pigs

### Effets du régime sur l'écosystème microbien gastro-intestinal et sur l'expression génétique de l'intestin chez les jeunes porcs

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#### Abstract

The microbial gastrointestinal ecosystem of young pigs is a dynamic system influenced by several factors, both external, like diet and stress, and host-related, like genetics and age. The gastrointestinal microbiota has long been considered an important factor affecting the overall health of the pig, and more recently, its impact on feed efficiency and even on behavior of the host are being intensively studied. Many dietary treatments based on different composition or addition of various additives, and feeding strategies have been investigated over the years with the aim of improving the animal's gut health. One goal has been to reduce the risk of pathogen colonization or stimulate the immune system, resulting in animals less susceptible to infections. Another goal has been to improve the gut's ability to digest feed ingredients and absorb the corresponding nutrients. In the search for the 'optimal' microbiota profile for best health and feed efficiency, research has moved from composition of microbiota to function of these populations, and the impact of changes of the gastrointestinal microbiota composition by dietary means on the host increasingly includes gene expression of the gut. The impact of some selected commonly used and effective dietary strategies on microbiota composition and function, and gene expression of the host will be presented and discussed.

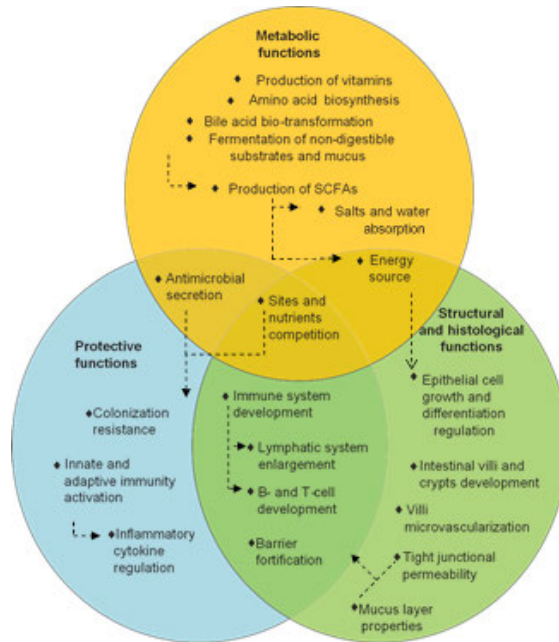
#### Résumé

L'écosystème microbien gastro-intestinal des jeunes porcs est un système dynamique influencé par plusieurs facteurs, qu'ils soient externes, comme le régime et le stress, ou propres à l'hôte, comme la génétique et l'âge. Le microbiote gastro-intestinal est considéré depuis longtemps comme un facteur important touchant la santé globale du porc et, plus récemment, son impact sur l'efficacité alimentaire et même sur le comportement de l'hôte a fait l'objet d'études intensives. Plusieurs traitements alimentaires – basés sur différentes compositions ou sur l'ajout de divers additifs – et stratégies d'alimentation ont fait l'objet d'investigations au fil des ans afin d'améliorer la santé de l'intestin des animaux. Un but était de réduire le risque de colonisation d'agents pathogènes ou stimuler le système immunitaire, rendant les animaux moins susceptibles aux infections. Un autre but était d'améliorer la capacité de l'intestin à digérer les ingrédients des aliments et absorber les nutriments correspondants. Pour dégager le profil « idéal » de microbiote pour la meilleure santé et la plus grande efficacité alimentaire, la recherche est passée de la composition du microbiote à la fonction de ces populations, et l'impact des changements de composition du microbiote gastro-

intestinal par des moyens alimentaires sur l'hôte inclut de plus en plus l'expression génétique de l'intestin. L'impact d'une sélection de stratégies alimentaires utilisées couramment sur la composition et la fonction du microbiote et sur l'expression génétique de l'hôte fera l'objet d'une présentation et d'une discussion.

## Introduction

It has long been recognized that the gastrointestinal microbiota exerts a big impact on the host that helps developing and maintaining a healthy gastrointestinal gut and extracting energy from the diet, with the beneficial impact this has on disease resistance, physiology, and nutrition of the host (Richards et al., 2005); (Anguita et al., 2006; Willing and Van Kessel, 2010). The multiple effects the gastrointestinal tract (**GI-tract**) microbiota has on the host can be classified within protective functions, metabolic functions, and structural and histological functions (Prakash et al., 2011) (**Figure 1**). These effects can be exerted via microbial metabolism with the resulting metabolites produced, the most intensively studied so far being lactic acid, short chain fatty acids, and some main metabolites from protein metabolism, but many others are also produced. These have effects both on the host and on other microbiota members. The effects can also be exerted via the microbial cells themselves, e.g., cell wall components that can stimulate processes in the host, help or reduce colonization, etc.



**Figure 1.** Main beneficial functions of the gastrointestinal microbiota. Circles represent the three principal classes of functions performed by the bacteria that inhabit the gut. Arrows represent causal relationships. **Abbreviation:** SCFA, short chain fatty acid. (From Prakash et al., 2011).

It has also been increasingly being made evident that the gut microbiome and the brain communicate in a bidirectional manner, with each possibly affecting the other's functions (Mohajeri et al., 2018). In this regard, in pig production, the possible influence of the brain-gut-microbiota axis on unwanted behaviours like tail biting has been considered, but research on this topic is still in its infancy (Brunberg et al., 2016). Although these aspects are beyond the purpose of the current manuscript, they illustrate the myriad of possible interactions with their corresponding consequences between the GI-tract microbiota and the host.

One important factor affecting the composition of the GI-tract microbiota, and thereby potentially, its function, is the diet, both diet composition and diet structure. In the following, main effects on microbial ecology and gene expression of selected dietary strategies shown to improve gut health of piglets will be described. This will be seen in the context of post-weaning diarrhoea (**PWD**), since this disease is in focus when dealing with young pigs. Also, a strategy being recently investigated to improve gut health and feed efficiency of pigs, namely faecal microbial transplantation, (FMT), will be shortly presented.

## Dietary strategies to improve the gastrointestinal microbial ecosystem and their impact on gene expression

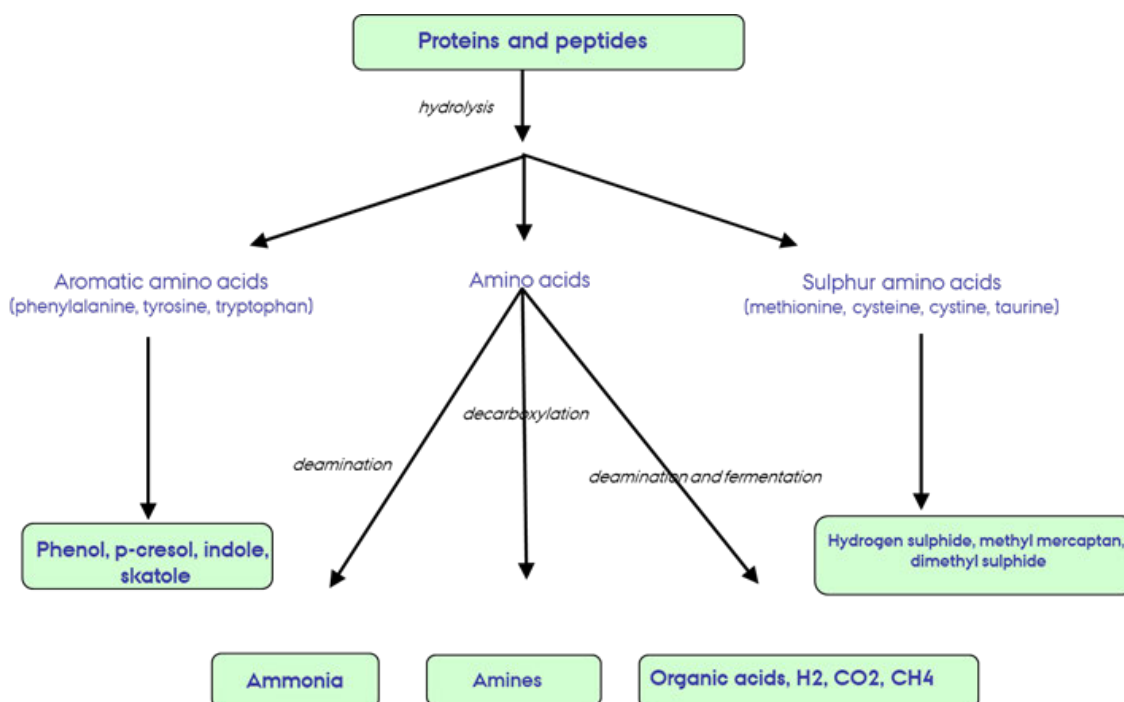
### *Dietary protein*

The impact of dietary protein on PWD, assumed to be exerted through changes in the GI-tract ecosystem, has long been recognized and intensively investigated (Porter and Kenworthy, 1969; Bikker et al., 2006) (Opapeju et al., 2009; Pieper et al., 2016). Feeding diets with reduced dietary protein level has repeatedly been shown to have a beneficial impact on PWD (Heo et al., 2008, 2009; Kim et al., 2011) and is recommended in situations of high PWD prevalence. However, concerns on a negative influence on growth performance is an issue. It seems, though, that low protein diets supplemented with crystalline amino acids that avoid amino acid deficiency/imbalance, do not impair growth performance (Heo et al., 2008; Kim et al., 2011). (Nyachoti et al., 2006) detected though impaired performance parameters when feeding piglets diets with too low CP levels (19% or lower) added crystalline amino acids. It has been shown that although low protein diets unsupplemented with amino acids have a negative short-term impact on growth performance, no such effect is observed when considering life span effects (Kim et al., 2011).

The hypothesized mode of action behind the positive effect of low protein diets is related to a reduction of protein microbial fermentation, especially in the proximal colon, but also in distal small intestine (Pieper et al., 2016). This results in a lower production, and thereby concentration, of potentially toxic metabolites, such as ammonia, biogenic amines, hydrogen sulphide, indoles, p-cresol, and phenols in the digesta, which at high concentrations (specific concentrations have not been established) are considered to have detrimental effects on the host through various mechanisms (Macfarlane and Macfarlane, 2012; Rist et al., 2013; Pieper et al., 2016; Gilbert et al., 2018), and thereby be an important causative factor of PWD. **Figure 2** illustrates the main metabolites from microbial proteolytic activity in the gut.

The expected reduction of metabolites from microbial proteolytic activity by feeding diets low in protein compared to high protein diets have been observed, mainly ammonia and biogenic amines and also branch chain fatty acids, in ileum or caecum/proximal colon with values of crude protein (CP) of 22-15% (Bikker et al., 2006); 23-17% (Nyachoti et al., 2006); 24-18% (Heo et al., 2008); 24-20% (Htoo et al., 2007); 22.5-17.5% (Opapeju et al., 2009); ~20-15% (Pieper et al., 2012); and ~26-18% (Pieper et al., 2014).

Although no impact on histopathology, feeding high-protein diets (20 vs. 15%) increased several indices associated with mucosal cell turnover and proinflammatory reactions, i.e., expression of PCNA, IL1b, IL6, MUC1, MUC2, and MUC20 (Pieper et al., 2012). These markers were associated with high concentrations of ammonia and biogenic amines. On the other hand, there was also an upregulation of antiinflammatory cytokines IL10 and TGFβ. These changes in cytokine expression were considered to be subclinical in nature, since no impairment of performance and health status was observed. High levels of CP can negatively affect epithelial barrier and histology but inconsistent results have been reported (Nyachoti et al., 2006; Hermes et al., 2009; Opapeju et al., 2009).



**Figure 2.** Main metabolites from microbial proteolytic activity in the gut.

The ratio fermentable protein to fermentable fiber is determinant for the substrates being fermented by the gut microbiota and thereby, the metabolites produced, that is, if fermentable fiber is available, the microbial proteolytic fermentation is reduced, and the amino acids are mainly incorporated in the microbial biomass (Yao et al., 2016). This factor is therefore obviously important to take into account when comparing studies or interpreting the results, and has specifically been investigated in several studies (Bikker et al., 2006; Hermes et al., 2009; Pieper et al., 2012; Pieper et al., 2014). In general, addition of fermentable carbohydrates reduces the levels of metabolites from proteolytic activity, e.g., ammonia in jejunum and colon (Bikker et al., 2006); ammonia and biogenic amines in caecum and colon (Pieper et al., 2012; Pieper et al., 2014); ammonia in cecum and colon (Awati et al., 2006). However, after (Pieper et al., 2016) had reviewed this aspect, considered that the reducing effect of fiber inclusion on microbial proteolytic metabolites can in general not reach the levels of low CP diets, and the resulting impact on gene expression as indicators of gut health have not been sufficiently affected. Further, the fermentability degree and site of fermentation leads to fibers affecting different metabolites and at different sites of the gut (Pieper et al., 2016). To illustrate the complexity of this system, (Hermes et al., 2009) registered a higher antibiotic treatment in piglets fed a low CP ~15%/high fiber diet than in a high CP (~19%)/high fiber diet. A higher relative contribution of endogenous protein substrates in the low CP diet compared to the high CP diet (Diether and Willing, 2019) or alterations in the gut epithelium due to too low CP levels could be speculated to contribute to the results. Further investigation is needed in order to establish the appropriate ratio between dietary CP and fiber level and fermentability.

Although being another disease, an aspect considered by (Diether and Willing, 2019) regarding human colorectal cancer could be speculated to some extent to be involved in diarrhoea in piglets, namely that increased fiber fermentation and short-chain fatty acid production appear to be protective against colorectal polyp development even when protein fermentation products are abundant. This would clearly need to be investigated.

Major amino acid fermenting bacteria in the GI-tract include proteolytic members of Fusobacteria; Proteobacteria, including *E. coli*; Firmicutes, including *C. perfringens*, *C. difficile*, *Selenomonas*, *Peptostreptococcus*; and Bacteroidetes, including *Veionella*, *Bacteroides* (Dai et al., 2011). Since some of these bacteria are candidate pathogens, it may partly explain why high protein diets in pigs have been associated with diarrhoea.

The discussion by (Diether and Willing, 2019) is very interesting in this regard. According to these authors, a newer approach using KEGG pathway analysis of annotated human gut bacterial genomes and probabilistic pathway construction shows that Proteobacteria possess the broadest gene coverage of amino-acid reactions, though only 9% are unique to this phylum. This can be explained by the high functional redundancy in the microbiome. When KEGG classification is performed on all predicted microbial reactions, the largest identified category is amino-acid metabolism. However, according to (Diether and Willing, 2019), due to the differences in substrate abundance, community membership, and species richness in different locations of the gut, it is important not only to establish which species are participating but also to examine how processes may differ in the small and large intestine.

The impact of dietary CP level on the gut microbial community composition of pigs has not been thoroughly studied. No effects on counts of *Lactobacilli* or coliforms/Enterobacteriaceae were observed in small intestine and/or colon (Bikker et al., 2006; Nyachoti et al., 2006; Hermes et al., 2009; Opapeju et al., 2009); no effect on total bacteria, Enterobacteriaceae, *Bacteroides* or *Cl. coccoides* group in proximal colon (Pieper 2012); no effect on T-RFLP profiles in colon) (Opapeju et al., 2009); increased numbers of *C. leptum* group (Pieper et al., 2012); increased lactobacilli to coliforms ratio (Wellock et al., 2006); no effect on coliforms in ileum or proximal colon and a decreased of lactobacilli in colon (Wellock et al., 2008) were observed when reducing the dietary CP level (See also the review by (Rist et al., 2013). After ETEC-challenge, the low CP diet increased the prevalence of bacteria in the order Clostridiales particularly family Lachnospiraceae and genus *Roseburia* and tended to reduce Clostridiaceae and genus *Clostridium* in colon digesta compared to the high CP (Opapeju et al., 2009).

In finisher pigs, (Fan et al., 2017) measured a sharp reduction in the abundance of *Cl. sensu stricto* (generally perceived as pathogenic in humans, (Rajilic-Stojanovic and de Vos, 2014) but a concomitant sharp increase of *Escherichia-Shigela* abundance in ileum by reducing the dietary CP level (16%, 13%, 10%). On the contrary, reducing CP increased the abundance of *Cl. sensu stricto* in colon. Reducing the CP level decreased the concentration of ammonia and biogenic amines in ileum and colon, but the epithelial integrity of the ileum (histology, tight junction proteins) seemed to be impaired by the lowest CP level (10%). Although the beneficial impact on microbiota composition was not clearly positive, it was concluded that a moderate reduction of CP (from 16% to 13%) indicated beneficial effects whereas a further reduction was detrimental.

Also in finishers, (Zhou et al., 2016) measured a sharp reduction in *Lactobacilli* abundance in the caecum and of *Streptococcus* and *Sarcina* in the colon by reducing the CP level (from 18% to 15%, growers; 16% to 13% finishers), concomitant with an increase of Peptostreptococcaceae in the caecum and a reduction in the colon. Lowering the CP level reduced the concentration of isobutyrate and isovalerate in caecum with no effect in colon.

In summary, these studies did not show a clear relationship between dietary CP level and a 'healthier' microbiota composition in the gut.

A parameter that can significantly influence the balance between proteolytic versus saccharolytic activity in the gut is transit time (Macfarlane and Macfarlane, 2012; Roager et al., 2016; Tottey et al., 2017) increased transit time leading to a higher proteolytic activity. Since increased transit time is seen in relation to weaning (Snoeck et al., 2004), this factor could also contribute to the increased concentration of metabolites from proteolytic bacterial activity observed in some weaners.

Although PWD is often related to proliferation of ETEC *E. coli*, it is known that non-infectious PWD occurs (Callesen et al., 2007). The aspects described above related to the deleterious effects of high concentration of metabolites from microbial proteolytic activity could contribute to explain the non-infectious type of PWD. But at the same time, high levels of CP exacerbate diarrhoea incidence in ETEC *E. coli* challenged newly weaned piglets (Wellock et al., 2008). This could be due to a higher luminal pH in the small intestine due to the buffer capacity of protein, which supports *E. coli* growth compared to more acidic conditions (Nyachoti et al., 2006), or to more substrates available for this proteolytic species (Heo et al., 2013).

### Organic acids

After the decision in many countries, including the EU in 2006, to phase out antimicrobial growth promoters, an intensive search for feeding alternatives to prevent PWD took place. Of many alternatives tested in Denmark, organic acids showed the most positive effect on growth performance. Some results are shown in (Table 1). The results of these and other studies also indicated that feed inclusion levels of around 1% might be needed to detect an effect. Several studies have also shown that organic acid addition results in reduced PWD (Tsilyiannis et al., 2001; Bosi et al., 2007; Partanen et al., 2007; Papatsiros et al., 2011; Callegari et al., 2016); and today, addition of organic acids to the feed of young pigs is a common practice in many countries. There is a high number of publications showing an antimicrobial effect of organic acids (Kirchgessner et al., 1992; Canibe et al., 2001; Knarreborg et al., 2002; Canibe et al., 2005), reviewed by (Suiryanrayna and Ramana, 2015) which has been considered a main mode of action for the effect of feeding diets added organic acids. There are, however, other mechanisms considered to contribute to the positive output. de Lange et al. (2010) summarized these possible mechanisms: (1) antimicrobial activity of non-dissociated organic acids; (2) lowering digesta pH, in particular in the stomach, aiding protein digestion; (3) lowering stomach emptying rate; (4) stimulating (pancreatic) enzyme production and activity in the small intestine; and (5) providing nutrients that are preferred by intestinal tissue thereby enhancing mucosal integrity and function. Further, organic acids may stimulate intermediary metabolism resulting in improved energy or protein/amino acid utilization (Kirchgessner and Roth, 1988); and may improve the absorption of minerals, particularly Ca and P, and influence the retention of minerals (Partanen and Mroz, 1999)

**Table 1.** Summary of trials conducted in Denmark to investigate the effect of various feed alternatives to antibiotic growth promoters on growth performance expressed as percentage change compared to control diets. From Maribo, H., SEGES, Denmark (Unpublished).

	Number of tri- als	Number of trials with significant effect	Effect on dai- ly gain,%	Effect on feed/gain, %
Antibiotics	13	8	+8,9	-3,5
Acids/Salts	53	16	+5,1	-1,2
<i>Acids/salts with significant effect</i>	<i>16</i>	<i>16</i>	<i>+9,4</i>	<i>-3,8</i>
Probiotics	17	1	+2,0	-1,9
Plant extracts and aromas	27	2	+2,2	-1,1
Enzymes	9	0	+2,1	-0,0
Oligosaccharides	7	1	+2,3	-1,2

The antimicrobial ability of different organic acids is different (Knarreborg et al., 2002) and is related to the reduction of pH, as well as their ability to dissociate, which is determined by the  $pK_a$ -value of the respective acid, and the pH of the surrounding milieu (Cherrington et al., 1991; Russell, 1992). That is, an antimicrobial effect can be seen without a pH reduction, for example, when added as a salt (Canibe et al., 2001). On the other hand, the results of (Canibe et al., 2005) clearly showed the impact of formic acid (at high levels, 1.8%) on pH of the gastric digesta. Also, what is worth noticing here is that the pH was maintained at low levels, i.e., below 4, at all times when measured from feeding and to ~8h post-feeding.

From an initial focus on the antimicrobial effect and protein digestibility of organic acids, more recent studies have attempted to investigate other possible response parameters. The weaning process often causes inflammatory responses and impairment of the epithelial barrier, leading to high permeability (Gresse et al., 2017). Paracellular absorption of molecules that would not traspass a healthy barrier and translocation of pathogens can thus occur (Gresse et al., 2017). Therefore, studies have looked at the direct or indirect impact of organic acids on parameters related to epithelial barrier and inflammatory response.

(Ferrara et al., 2017) hypothesized that short and medium chain fatty acids would affect gut morphology (villus length and crypt depth of the jejunum) and the local intestinal immune system (intra-epithelial lymphocytes) in weaned piglets due to the effects on the intestinal microbiota and their role as energy source for the epithelial cells. A mixture of fumaric acid (0.41%) and lactic acid (0.32%), or the combination of these with caprylic (0.15%) and capric acid (0.15%) was investigated and compared to a control. The supplementation of the acids did not significantly affect morphometric data. On the other hand, short chain fatty acids addition significantly increased

the quantity of CD2<sup>+</sup> CD8<sup>+</sup>  $\gamma\delta$  T cells in the jejunum epithelium, which might have a beneficial effect on the local immunity by conferring protection to infectious diseases. (Pu et al., 2018) studied the impact of 0,3% benzoic acid combined with *Bacillus coagulans* or with oregano oil in ETEC challenged piglets. They concluded that the treatments could improve growth performance and alleviate diarrhoea of the piglets via improving intestinal mucosal barrier integrity, which was possibly associated with the improvement of intestinal microbiota and the reduction of proinflammatory cytokines production via inhibition of TLR4 and NOD2 signaling pathways.

Feeding piglets a diet added a mixture (0.3%) of organic acids (calcium formate, calcium lactate, citric acid) and medium chain fatty acids, tested as an alternative to zinc oxide, resulted in higher expression of the peptide and amino acid transport genes CAT2, EAAT3 in the jejunum; lower expression of TNF- $\alpha$  and higher of TNF- $\beta$  in jejunum; lower plasma concentration of TNF- $\alpha$  and higher of IgG; and higher number of lactobacilli in ileum and rectum (Kuang et al., 2015). Due to the reported impact of lactic acid bacteria on the immune response, the effect of the additives on cytokine expression was considered to be associated to the increased proliferation of lactobacilli following organic acids consumption.

(Grilli et al., 2015) aimed at investigating whether a combination of organic acids (sorbic acid, citric acid) and botanicals (thymol and vanillin) had an impact on intestinal health and mucosa barrier function at weaning; and whether the effect was a direct effect, i.e., non-microbiota mediated, on intestinal epithelial cells. Although the results were not too clear, it was concluded that organic acids + botanicals seemed to positively modulate the inflammatory stress in the intestinal epithelium by reducing pro-inflammatory stimulus, rather than enhancing the anti-inflammatory response. Studies with Caco-2 cells to isolate the possible impact of the treatment from the effect on the microbiota showed an increased trans-epithelial resistance of the cells by the organic acid-botanical mixture, indicating a direct role in ameliorating epithelial integrity via a microbiota-independent pathway. Although the mechanism behind this effect is uncertain, it was speculated to be related to effects of organic acids in regulating IGF gene expression and secretion or as energy substrate for the cells. Using an ex-vivo model by incubating jejunal tissue from piglets with benzoic acid (Silveira et al., 2018) reported no effect on gene expression of GLUT2, IGF-1, or RelA/p65, but measured a down-regulation of the gene expression of the glucose transporter SGLT1. The significance of these results could not be established, though.

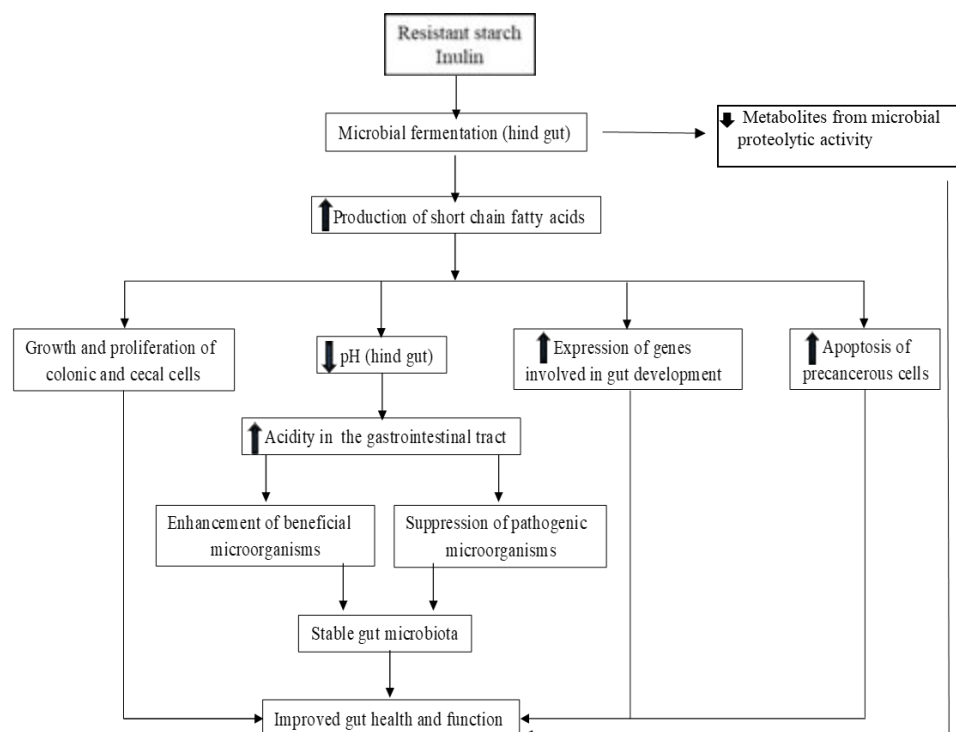
It can be summarized that organic acids have long been shown to positively affect health and growth parameters in young pigs mainly considered to be due to their antimicrobial effect and to the impact on protein digestibility. A few studies have intended to identify other modes of action related to epithelial integrity and immune response around weaning with results showing some effects. There are though few studies and some of them have used combinations of acids with other components, which makes the interpretation more difficult. Moreover, although some efforts have been made to separate the possible effects of organic acids through the known impact on the microbiota from more direct effects of the acids, this issue has not been elucidated yet.

### ***Resistant starch and inulin***

Another approach to influence pig health is the dietary inclusion of specific high fermentable dietary fiber components to improve gut health, being inulin and resistant starch (**RS**) some of the most widely studied.

As resistant starch escapes digestion in the small intestine, it serves as substrate for fermentation in the hindgut and has been associated with, among other effects, increased short-chain fatty acids (SCFA) production and stimulation of amylolytic and butyrogenic bacteria (Birt et al., 2013). An stimulating effect of RS on butyrate production in the hindgut, through a prebiotic effect, is considered in the literature to be the main mode of action behind the beneficial impact of feeding RS on health (reviewed by (Guilloteau et al., 2010); (Regassa and Nyachoti, 2018)). **Figure 3** illustrates the proposed pathways through which resistant starch (and inulin as discussed below) exerts its effects on the host's health. Inulin-type fructans are also resistant to hydrolysis by enzymes in the small intestine, but are rapidly fermented and stimulate saccharolytic bacteria including bifidobacteria and lactobacilli (Patterson et al., 2010; Jensen et al., 2011; Tran et al., 2016), reviewed by (Kozłowska et al., 2016). Inulin is, therefore, defined as a prebiotic (Zimmermann et al., 2001). Both dietary fiber components are therefore considered to enhance gut health by providing energy sources to the colonocytes, supporting the immune system, preventing proliferation of pathogens, etc.

There are numerous studies and reviews in the literature on the impact of RS (resistant granules) and inulin on pigs, which will not be described or discussed here. Instead, two recent meta-analyses by Metzler-Zebeli on inulin (Metzler-Zebeli et al., 2017) and RS2 (Metzler-Zebeli et al., 2019) will be briefly presented



**Figure 3.** Illustration of how resistant starch improves gut health (Modified from Regassa et al., 2018)

The meta-analysis on inulin (Metzler-Zebeli et al., 2017) included studies with dietary inulin levels ranging from 0.1 to 25.8%, whereby the median and mean inulin levels were 0.1–2% and 3–4%, respectively. The analysis carried out on studies dealing with weaned, growing, and finishing pigs, showed that few of the investigated fermentation response variables were influenced by dietary inulin. In weaned pigs, gastric pH showed a negative linear relationship with increasing dietary inulin levels, a dietary inclusion level of 3% would decrease gastric pH by 0.12 units. The digesta pH in ileum, cecum, colon, and feces was not affected by the dietary inulin level. The ileal lactate concentration tended to increase with more inulin in the diet. In contrast, there was a small negative relationship between the cecal concentration of acetate and increasing dietary inulin levels. Fermentation metabolites in colonic digesta and feces, in turn, were not influenced by the dietary inulin level.

The ileal lactobacilli and bifidobacteria numbers were not affected by dietary inulin level. Likewise, an increasing dietary inulin level from 0 to 20% did not modify the absolute abundance of lactobacilli in colonic digesta. By contrast, higher dietary inulin levels lowered the colonic abundance of bifidobacteria and enterobacteria, the inhibiting effect on enterobacteria was twice as strong as for bifidobacteria. In feces, increasing inulin levels reduced the abundance of lactobacilli, whereas bifidobacteria tended to be slightly enhanced by dietary inulin. Increasing dietary inulin levels reduced the absolute enterobacteria abundance and the abundance of *E. coli* in feces. It was concluded that the study supported a stimulatory effect of dietary inulin on gastric acid secretion, which may be favorable GI-tract health in weaned pigs. However, due to limiting information provided in the original studies, like dietary fructans or fibers, low numbers of observations and low inulin levels, relationships should be regarded as trends.

The meta-analysis on RS2 (Metzler-Zebeli et al., 2019) showed negative relationships between dietary RS and pH in the large intestine, with a stronger effect in the mid and distal colon and feces. Increasing RS levels, however, did not affect total or individual SCFA concentrations in ileal, cecal, colonic digesta or feces, but enhanced the molar proportion of propionate in mid-colon and reduced those of acetate in mid-colon and of butyrate in mid- and distal colon. A minimum of 20% of dietary RS would be needed to elevate the molar propionate proportion by 5% in mid-colonic digesta. In feces, increasing RS levels promoted fecal lactobacilli and bifidobacteria, whereby the slope showed the need for a minimal RS level of 10% for a 0.5 log unit-increase in their abundance. No effect on faecal Enterobacteriaceae or total bacteria in colon was detected. Best-fit equations further supported that a longer experimental period increased fecal lactobacilli but decreased fecal bifidobacteria. The authors could conclude that dietary RS2 seems to effectively decrease digesta pH throughout the large intestine and increase lactic acid-producing bacteria in feces of pigs. Further, to achieve these physiologically relevant changes, dietary RS should surpass 10% to 15%.

Although being aware of the limitations this type of studies might have, it is surprising that, two dietary ingredients considered amongst the most effective to beneficially impact pigs' health did not show stronger effects. An aspect worth noticing in both meta-analyses is the (high) level needed to obtain the searched beneficial effects.

### ***Faecal microbiota transplantation***

The afore described dietary strategies are examples of the search for strategies to prevent disease by manipulating the GI-tract microbiota. Only recently has faecal microbiota transplantation

(FMT) been investigated for the purpose of GI-tract microbiome manipulation in pigs with the aim of improving phenotypes in these animals. Some studies have examined various parameters related to intestinal health, including intestinal development, the intestinal epithelial barrier, and microbiota composition (Hu et al., 2017; Cheng et al., 2018; Diao et al., 2018; Geng et al., 2018; Hu et al., 2018; Lin et al., 2018). Others have investigated the use of FMT as a possible strategy to improve feed efficiency of recipient pigs (McCormack et al., 2018), Canibe et al. (Unpublished).

The well known high efficacy of FMT to treat recurrent *Clostridium difficile* infections in humans, where ca. 90% resolution has been reported (Kassam et al., 2013; Choi and Cho, 2016), together with data from pigs relating gastrointestinal tract microbiota composition with growth performance (Ramayo-Caldas et al., 2016; McCormack et al., 2017) has most probably played an important role in the interest for this strategy in pig production to both prevent/treat disease and improve feed efficiency.

Regarding young pigs, a few number of studies have been published on the impact of FMT. (Hu et al., 2017; Cheng et al., 2018) used Jinhua pigs as donor pigs, which according to the authors are considered to be more resistant to ETEC *E. coli* F4. The authors concluded that FMT changed the population structure of intestinal microbiota, which contributed to the improvement of intestinal morphology, the development of the intestinal mucosal barrier, and innate immunity in recipient piglets (Hu et al., 2017). Further, FMT triggered mucosal protective autophagy and thereby protected the integrity of the intestinal barrier (Cheng et al., 2018). (Geng et al., 2018) investigated FMT as a strategy to change the GI-tract microbiota of the recipients in a way that would maintain intestinal homeostasis by regulating mucosal integrity and immune responses. According to the authors, FMT reduced the susceptibility to LPS-induced destruction of epithelial integrity and severe inflammatory response.

Following a similar approach of using donors considered to be more resistant to disease, (Hu et al., 2018) used Congjiang miniature piglets as donors. An impact of the microbiota composition of the donors was measured as a consequence of the FMT and a reduction of diarrhoea incidence in the recipients was reported after FMT. Specific bacterial species were proposed to be involved in the beneficial effects. Very few replicates were used in this study, though.

When (Diao et al., 2018) transplanted feces from three breeds, Tibetan, Yorkshire, and Rongchang. Transplantation of fecal microbiota from Yorkshire and Rongchang pigs to Duroc-Landrace-Yorkshire suckling piglets adversely affected the gut microbiota balance and intestinal health, related to epithelial barrier, intestinal development, digestion, and absorption; whereas transplantation of the fecal microbiota derived from the Tibetan pigs was considered not to have negative effects but promote absorption enzymes activity in the recipient suckling piglets.

(McCormack et al., 2018) had another approach, which was based on improving feed efficiency in the recipients via beneficial modulation of the intestinal microbiota by transplanting feces from highly feed-efficient pigs to sows and/or their offspring. They observed changes in the microbiota community composition and function but the data showed that FMT resulted in reduced body weight in the piglets, which was hypothesized to be related to a possible negative impact of the FMT on absorptive capacity and intestinal health. Also with the aim of using FMT to improve feed efficiency in the donors, Canibe et al (Unpublished) transplanted colon digesta from pigs given a

control diet, or control added 170 ppm copper as copper sulphate, 40 ppm tylosin, or 1% benzoic acid. Preliminary results showed that the microbiota did not established in the recipients when measured 10 weeks after FMT was practiced.

To investigate whether FMT could prevent diarrhoea in piglets (Cheng et al., 2018) challenged piglets with ETEC *E. coli* F4, and according to the authors, the results indicated that FMT enhanced the epithelial barrier and protected the intestinal epithelium after *E. coli* K88 infection.

It could be summarized that the studies conducted so far on FMT to improve feed efficiency or gut health in piglets show inconsistent results, which can be due to the numerous factors that can affect the output, e.g, donor microbiota, age of donor, age of recipient, time of transplantation, preparation of the material to be transplanted, etc. At the same time, the results show the potential of this technique, also taking into account the positive results from humans. On the other hand, it is evident that FMT poses a high health risk, since the microbiota of the donor cannot be screened for all possible pathogenic microorganisms present. Also, it is not known whether all recipients can cope with the microbiota even of a theoretically completely healthy donor, which could be compared to organ transplantation in humans. But while investigating more targeted/refined strategies to give specific members of the microbiota to a donor that improve a certain phenotype, it is worth investigating FMT further, and in fact, FMT can be a way of identifying such specific bacteria/consortia of bacteria.

## Conclusions

Numerous dietary strategies have been and continue being studied to manipulate the gastrointestinal ecology of young pigs, to, in that way, improve their ability to cope with stressful situations, weaning being the most studied. The main aim is to improve the robustness of the animals so that their resistance to disease increases, and thereby, the need for antibiotic treatment is reduced, as well as their growth performance and welfare are improved.

The mechanism of action of many strategies are partially elucidated but, when put into practice under different conditions, varying outputs are obtained. This is probably due to the high number of factors that affect the results, from dose of the item tested/difference in level between experimental and control diets, to the animals' health, etc., which when combined in different ways, make prediction of the responses more difficult

In order to further investigate the mode of action behind the observed effects, in more recent years, dietary strategies that have been shown to have beneficial effects, like those presented here, are being studied using supplementary response parameters. These include sequencing techniques to investigate the microbiota composition and function; metabolomics in digesta, plasma or urine; gene expression in the host, proteomics, etc. Also newer strategies are being investigated, FMT being an example of these, which clearly need to be further investigated.

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## **In Ovo and Neonatal Nutrition in Poultry**

### **Nutrition de l'embryon et du nouveau-né chez la volaille**

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#### **Abstract**

Innovation in breeding and genetic selection for increased growth rate and meat yield has dramatically advanced the production efficiency of poultry during the last 50 years, and this trend is expected to continue well into the future. Now, the period of embryonic and neonatal development is approaching 50% of the productive life of modern broilers, turkeys, and ducks. Although genetic selection determines genetic potential, nutrition and management influences the expression of genetic potential and drives economically important performance traits. Incubation and neonatal distress have lasting adverse effects on performance and welfare, but perinatal nutrition and management has not kept pace with advances in other aspects of poultry production. However, as in ovo and early feeding technologies develop, opportunities to alleviate early nutrition and developmental constraints and program gene expression become increasingly feasible. In poultry, epigenetic programming, which allow an animal to metabolically or physiologically adapt to specific dietary or environmental conditions, can occur during two critical periods: when embryo consumes the amniotic fluid prior to hatch, and when the chicks absorb residual yolk and feed during the first few days after hatch. This paper discusses implications of nutritional and physiological stress of embryos and hatchlings on their epigenetic response, and how it can be modified by perinatal nutrition, including amniotic fluid supplementation by in ovo feeding and early feeding technologies. Epigenetic and adaptive conditioning of neonatal nutrition will also be discussed in the context of a “programmed nutrition” strategy to increase production efficiency and meat quality.

#### **Résumé**

L'innovation en matière de reproduction et de sélection génétique pour augmenter le taux de croissance et le rendement de la viande ont favorisé considérablement l'efficacité de la production de volailles depuis 50 ans et l'on prévoit que cette tendance se poursuivra loin dans le futur. Maintenant, la période de développement embryonnaire et néonatal approche 50 % de la vie productive des poulets à griller, dindes et canards modernes. Même si la sélection génétique détermine le potentiel génétique, la nutrition et la gestion influencent l'expression du potentiel génétique et entraîne des traits de rendement économiquement importants. L'incubation et la détresse néonatale ont des effets nuisibles durables sur le rendement et le bien-être, mais la nutrition et la gestion périnatales n'ont pas suivi les percées dans d'autres aspects de la production

de volailles. Toutefois, à mesure que se développent les technologies d'alimentation in ovo et en bas âge, les opportunités de redresser les contraintes de la nutrition en bas âge et du développement et de programmer l'expression génétique deviennent de plus en plus réalisables. Chez les volailles, la programmation épigénétique, qui permet à un animal de s'adapter métaboliquement ou physiologiquement à des conditions alimentaires ou environnementales données, peut se produire durant deux périodes critiques : quand l'embryon consomme le liquide amniotique avant l'éclosion; et quand les poussins absorbent le jaune résiduel et s'alimentent les premiers jours après leur éclosion. Cet article traite des implications du stress nutritionnel et physiologique des embryons et des poussins éclos sur leur réponse épigénétique et sur la façon dont elle peut être modifiée par la nutrition périnatale, incluant par la supplémentation du liquide amniotique avec des technologies d'alimentation in ovo et d'alimentation en bas âge. Le conditionnement épigénétique et adaptatif de la nutrition néonatale fera aussi l'objet d'une discussion dans le contexte d'une stratégie de « nutrition programmée » visant à augmenter l'efficacité de la production et la qualité de la viande.

## Introduction

Modern agriculture constantly strives to maximize biological performance of food production in an effort to optimize economic efficiency, profit potential, and sustainability. Commercial poultry production is among the most efficient and progressively successful of all food production sectors. What factors does it take for continued success in efficient poultry production? It takes the right genetics, combined with optimum health and management practices, and an optimized nutrition and feeding program. Efficiency and sustainability depends on the ability of a poultry production company to achieve competitive production indicators, including average daily gain, days to market weight, feed (caloric) conversion, livability, flock uniformity, and processing yields. However, profitability largely depends on how well a poultry production company meets consumer demand. Consumers want wholesome, safe, and affordable food. They want poultry products that look good, and are enjoyable to eat. Moreover, the most affluent consumers also want to buy their food from companies that excel in environmental stewardship and animal welfare. After proper management of commercial genetic stock, nutrition and feed is the most variable component of economic efficiency and profitability, as it represents 70 to 80% of live production costs.

Genetic selection is continually changing the “playing field” of production potential for the poultry industry; but it is the expression of this genetic potential that drives growth performance, health, and ultimately the profitability of poultry production. Growth performance and meat yield has improved linearly by about 1% each year, and 85% of this improvement is attributed to genetic selection of broilers (Havenstein et al., 2003) and turkeys (Havenstein et al., 2007). One may argue that nutritional advancements have not kept pace with genetic selection as metabolic disorders and apparent nutritional deficiencies continue to arise, which mandate diet formulation constraints to be updated. However, the time has come to close this pace gap as we learn to harness the power of perinatal nutritional imprinting and adaptive conditioning to program the expression of genes associated with socioeconomically important traits. There is now growing evidence that nutrition and environmental stimuli of parent stock and their progeny during the perinatal period may literally program how an animal's genes are expressed as an adaptive response to increase the chances of survival. This new science of “gene expression programming” is Epigenetics; it is the inheritance of information on the basis of gene expression, or inherited adaptation.

## Epigenetic or Adaptive Conditioning

Epigenetics literally means “on genes”, and refers to all modifications to genes other than changes in the DNA sequence itself. DNA within each cell is wrapped around proteins called histones. Both the DNA and histones are covered with chemical tags, to form what is called the epigenome. These chemical tags react to signals to the outside world, such as diet and stress. Some parts of the epigenome are wrapped and unreadable, and other parts are relaxed and readable for expression. Epigenetic imprinting of genes occurs most often by differential methylation of DNA at the promoter regions of specific genes that can permanently modulate an organism’s adaptive response to adverse stimuli during critical periods of development. Particularly, early-life programming can turn on “Thrifty” genes that permanently reprogram normal physiological responses to survive environmental stressors, including moderate nutrient deficiency, and thus increase the chances of passing on their genes to the next generation. Evidence for epigenetic programming is demonstrated by swarming locusts: the swarming phenotype is environmentally influenced by drought conditions and the trait is passed onto the next generation until the population finds better conditions.

Transgenerational epigenetic or adaptive conditioning may explain some of the blessings and curses observed as a result of our system of commercial poultry production. Consider how we manage the weight of broiler or turkey breeders before and during egg production: this is during the critical epigenetic period of gametogenesis. Broiler breeder nutrition and feeding management likely has an important epigenetic effect on progeny. Consider how we manage and incubate commercial hatching eggs: this is during the critical epigenetic period of *de novo* methylation of somatic cells in the embryo. Environmental conditions (*i.e.* temperature and oxygen concentration) in the incubator may program epigenetic responses that affect subsequent metabolism. Consider how we manage chicks during the first few days after hatch. Feeding behavior, nutrition, and brooding conditions can affect metabolism and the development of breast muscle, the skeleton, and immune system.

## In Ovo Feeding Jump-Starts Perinatal Development

Phenotypic characteristics that are programmed or imprinted to succeed in its given environment and diet happen most effectively when the animal is young, and it is the first few meals that usually make the difference. For example, all honeybees are genetically similar, but what predestines a bee to become a worker or a queen is what the larvae are fed. Likewise, poultry may be programmed to succeed with the desired phenotypic traits by nutritional modification during the perinatal period: the 3 days before hatch and the 3 days after hatch. The chick’s first meal occurs when it imbibes the amnion prior to hatch, and so this is the first opportunity for nutritional programming. By in ovo feeding (Uni and Ferket, 2003; US Patent No. 6,5692,878), nutrient balance and key metabolic co-factors of the amnion meal can be modified and influence subsequent phenotypic traits of economic importance for the poultry industry.

The benefits of in ovo feeding on early growth and development of broilers and turkeys have been demonstrated by several experiments in our laboratory (Uni and Ferket, 2004). Now after 15 years of research by scientists all over the world, the effects of in ovo feeding of a large variety of nutrients and non-nutrient supplements have been demonstrated (Table 1). In ovo feeding

has increased hatchling weights by 3% to 7% ( $P < .05$ ) over controls, and this advantage is often sustained at least until 14 days post-hatch. The degree of response to *in ovo* feeding may depend upon genetics, breeder hen age, egg size, and incubation conditions (i.e. the epigenotype). Above all, IOF solution formulation has the most profound effect on the neonate. Positive effects have been observed with IOF solutions containing NaCl, sucrose, maltose, and dextrin (Uni and Ferket, 2004; Uni et al., 2005),  $\beta$ -hydroxy- $\beta$ -methyl butyrate, egg white protein, and carbohydrate (Foye et al., 2006a), Arginine (Foye et al., 2007), zinc-methionine (Tako et al., 2005), butyric acid (Salmanzadeh et al., 2015), IGF-1 (Liu et al., 2012), and L-glutamine (Shafey et al., 2013). In addition to the increased body weights typically observed at hatch, the positive effects of *in ovo* feeding may include increased hatchability (Uni and Ferket, 2004; Uni et al., 2005); advanced morphometric development of the intestinal tract (Uni and Ferket, 2004; Tako et al., 2004) and mucin barrier (Smirnov et al., 2006); enhanced expression of genes for brush boarder enzymes (sucrase-isomaltase, leucine aminopeptidase) and their biological activities, along with enhanced expression of nutrient transporters, SGLT-1, PEPT-1, and NaK ATPase (Tako et al., 2005; Foye et al., 2007); increased liver glycogen status (Uni and Ferket, 2004; Uni et al., 2005; Tako et al., 2004; Foye et al., 2006a); enhanced feed intake initiation behavior (de Oliveira, 2007); increased breast muscle size at hatch (Uni et al., 2005; Foye et al., 2006a), breast muscle growth and meat yield (Kornasio et al., 2011), and improved skeletal development (Yair et al., 2015). *In ovo* feeding clearly advances the digestive capacity, energy status, and development of critical tissues of the neonate by about 2 days at the time of hatch. Using scanning electron microscopy, Bohórquez *et al.* (2008) observed that *in ovo* feeding significantly increased functional maturity and mucus secretion of goblet cells of villi of ileum and ceca of turkey poults. Associated with these goblet cells was the colonization of lactobacilli. Therefore, *in ovo* feeding may help improve the colonization resistance of enteric pathogens of neonatal chicks and poults. Several researchers have demonstrated the benefits of *in ovo* delivery of prebiotics and synbiotics (Madej et al., 2015; Siwek et al., 2018;), probiotics (Pender et al., 2017; de Oliveira et al., ; Peebles, 2018) Based on the rapidly growing number of peer-reviewed publications from around the world, *in ovo* feeding consistently shows promising benefits, especially if applications can be done without compromising hatchability (Kadam et al., 2013; Cardeal et al., 2015; Retes et al., 2018; Peebles, 2018).

*In ovo* feeding offers promise of sustaining the progress in production efficiency and welfare of commercial poultry. Although selection for fast growth rate and meat yield may favor the modern broiler to become a more altricial, proper early nutrition and *in ovo* feeding may help these birds adapt to a carbohydrate-based diet and metabolism typical of a precocial bird at hatch. Our original research on *in ovo* feeding has established a new science of neonatal nutrition that many other scientists are now pursuing. As a result, we are all gaining greater understanding of the developmental transition from embryo to a juvenile bird. Now more work on *in ovo* feeding application technology and hatchery logistics must be done before *in ovo* feeding can be widely adopted for commercial practice.

## Potential of Post-Hatch Nutrition on Nutritional Imprinting

The first few days post-hatch is the second part of the perinatal period that can imprint production traits by adaptive conditioning of gene expression. Chicks can be imprinted to enhance their tolerance to immunological, environmental, or oxidative stress. Nutritional programming during

the perinatal period can also influence energy and mineral utilization or requirement, while other bioactive dietary components may “program” enteric microflora colonization that affect gut health and food safety. For example, Yan et al. (2005) reported that conditioning broilers fed a diet low in calcium and phosphorus for 90 hours post-hatch improves intestinal calcium and phosphorus absorption at 32 days of age, and increases the expression of the gene for the mineral transporter protein throughout the life of the bird. Angel and Ashwell (2008) demonstrated that broilers fed a moderately deficient conditioning diet for the first 90 hour post-hatch were more tolerant to a P-deficient grower and finisher diet, but they were also heavier, had better feed conversion, and they had higher tibia ash and P retention. The work of Angel and Ashwell demonstrate that epigenetic imprinting and nutritional adaptation to low dietary Ca and P is indeed possible and likely for other minerals as well.

Based on the concepts of epigenetics, imprinting, and adaptive conditioning presented above, several experiments has been done to test various nutritional programming strategies at the Alltech-University of Kentucky Nutrition Research Alliance Coldstream Farm and Alltech’s Center for Animal Nutrigenomics and Applied Animal Nutrition. By evaluating the expression patterns of key functional gene groups, dietary amounts of nutrients that affect homeostatic balance were discovered to depend on the form of the nutrient, levels of and interactions among nutrients, and the timing of administration. Feeding chicks a specifically-formulated diet during the first 72 hours post-hatch has been developed to “condition” the gut for better nutrient utilization and program metabolism that ultimately affects production efficiency, carcass composition, and meat quality. Chicks that have been fed the appropriate conditioning diet, followed by a complementary growing and finishing diet, have improved growth performance and feed efficiency through to market age, and over 70% higher calcium and phosphorus digestion than controls. A programmed nutrition strategy can literally change the nutrient requirement and production efficiency, and may yield a response greater than any single feed additive on the market. Not only can programmed nutrition increase production efficiency that is so important to poultry producers, there is evidence that it improves the meat quality consumers demand, which yields greater potential profits from the poultry products produced. Broilers that have been raised on a programmed nutrition strategy have reduced carcass fat and produce breast meat that has more appealing color, less drip losses during storage, improved oxidative stability, and lower cooking losses.

Although feeding broilers a special nutritional conditioning diet for just 72 hours after hatch presents great opportunities, it is logistically difficult to accomplish in practice using current production systems. Moreover, variation in the time and stress exposure between hatch-pull and placement will affect the effectiveness of the 3-day nutritional conditioning period. However, recent advancements in hatchery technology offers a practical means to deliver specially formulated diets during the first 2 or 3 days post-hatch in the controlled environment of a hatchery. The hatchery of the future will be a place that will do much more than simply hatch and vaccinate chicks: it will also be the place where the chicks will be conditioned better tolerate the challenges of life, and be programmed for optimum nutrient efficiency. Nutritional science is no longer a matter of supplying minimally required nutrients in the ideal balance to achieve desired production and welfare goals. We now know that nutrition is a process that can be programmed to succeed by strategic perinatal diet manipulation by in ovo and post-hatch feeding.

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## Precision Feeding of Gestating First Parity Sows Improves Sow Body Weight Gain in Late Gestation

### L'alimentation de précision des truies primipares gestantes améliore le gain de poids corporel en fin de gestation

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#### Abstract

Gestating sows experience varying nutrient and energy requirements throughout gestation. Failing to adequately meet these changing requirements can lead to sub-optimal body condition, reduced reproductive performance, and diminished sow longevity. The objective was to determine the effects of precisely meeting estimated (daily) energy and Lys requirement for first parity gestating sows on sow body weight and back fat changes throughout gestation. Ninety, first parity sows were randomly assigned to a precision (PF; n=49) or control (CON; n=41) feeding program between day 2 and 9 of gestation and housed in group-pens equipped with electronic sow feeders (ESF) capable of blending 2 diets. The PF sows received unique daily blends of two isocaloric diets (2518 kcal/kg NE; 0.80 and 0.20% SID Lys, respectively) while the CON sows received 2.2kg of a static blend of the diets to achieve 0.56% SID Lys. Sow body weights were measured weekly, back fat thickness was determined via ultrasound in early (~d5), mid (~d60), and late (~d110) gestation, and litter characteristics (e.g. born alive, birth weight) were recorded after farrowing. The PF sows had greater body weight gains in late gestation (i.e. when nutrient requirements are greatest; between day 60 and 110) versus CON sows (859 vs 484 g/d;  $P<0.05$ ). Back fat thickness increased continuously for PF sows but decreased between day 60 and 110 of gestation for CON sows. The total amount of body weight gain during gestation and number of piglets born alive did not differ, but PF sows tended to have fewer stillbirths (0.24 vs 0.53;  $P=0.058$ ) and lower litter birth weights (15.0 vs 16.5 kg;  $P=0.09$ ) than CON sows. Therefore, energy, and likely amino acids, were limiting for the CON sows by late gestation as they sacrificed both back fat and body weight gain in order to support litter growth. The long-term consequences for both the sow and her offspring remain to be elucidated.

#### Résumé

Les besoins nutritifs et énergétiques des truies gestantes varient tout au long de la gestation. Ne pas répondre adéquatement à ces exigences variables peut se traduire par un état corporel sous-optimal, une moins bonne performance reproductive et une diminution de la longévité des truies. L'objectif était de déterminer l'effet sur le poids corporel des truies et la variation du gras dorsal pendant la gestation si on comblait exactement les besoins estimés (quotidiens) en énergie et en Lys des truies primipares gestantes. Quarante-deux truies primipares ont été assignées au hasard à un programme d'alimentation de précision (PF; n=49) ou témoin (CON; n=41) entre le jour 2 et

le jour 9 de la gestation et logées dans des enclos collectifs équipés de mangeoires électroniques (ESF) capables de mélanger deux rations. Les truies PF ont reçu des mélanges quotidiens uniques de deux rations isocaloriques (2518 kcal/kg ÉN; 0,80 et 0,20 % Lys NDI, respectivement), tandis que les truies du traitement CON ont reçu 2,2 kg d'un mélange statique des rations pour atteindre 0,56 % Lys MDI. Le poids corporel des truies a été mesuré chaque semaine, l'épaisseur du gras dorsal a été déterminée par échographie au début (~j5), au milieu (~j60) et à la fin (~j110) de la gestation et les caractéristiques des portées (p. ex., nombre de nés vivants, poids à la naissance) ont été enregistrées après la mise-bas. Les truies PF ont présenté des gains de poids supérieurs en fin de gestation (c.-à-d., lorsque les besoins en nutriments sont les plus grands, entre le j60 et le j110) à ceux des truies CON (859 vs 484 g/j;  $P < 0,05$ ). L'épaisseur du gras dorsal a augmenté continuellement chez les truies PF et a diminué entre le 60e et le 110e jour de gestation chez les truies CON. Aucune différence n'a été enregistrée pour le gain de poids corporel total réalisé pendant la gestation et le nombre de porcelets nés vivants, mais les truies PF ont eu tendance à présenter moins de mortinatalités (0,24 vs 0,53;  $P = 0,058$ ) et un poids de portée inférieur (15,0 vs 16,5 kg ;  $P = 0,09$ ) que les truies CON. Par conséquent, l'énergie et probablement les acides aminés ont été des facteurs limitants chez les truies CON vers la fin de la gestation, période où elles ont dû sacrifier à la fois la production de gras dorsal et de gain de poids corporel afin de soutenir les portées. Les conséquences à long terme pour la truie et sa progéniture restent à élucider.

## Introduction

Gestating sows experience varying nutrient and energy requirements throughout gestation and across parities, which are largely driven by the growth of the products of conception and maternal maintenance and growth (i.e. for immature sows) requirements. For energy and amino acid partitioning, sows first prioritize maintenance requirements, followed by growth of the products of conception (i.e. fetus, mammary gland, uterus, placenta and associated fluids) and deposition toward maternal stores (i.e. maternal protein and lipid deposition; NRC, 2012; Farmer, 2015). Each of the pregnancy-associated tissues have a unique amino acid profile and the growth of each occurs at vastly different rates and times during gestation. For example, around day 40 of gestation the placenta and fluids begin to develop rapidly but growth plateaus at around 70 days. Conversely, exponential fetal growth begins at approximately 60 days of gestation and continues until the end of gestation (NRC, 2012; Farmer, 2015).

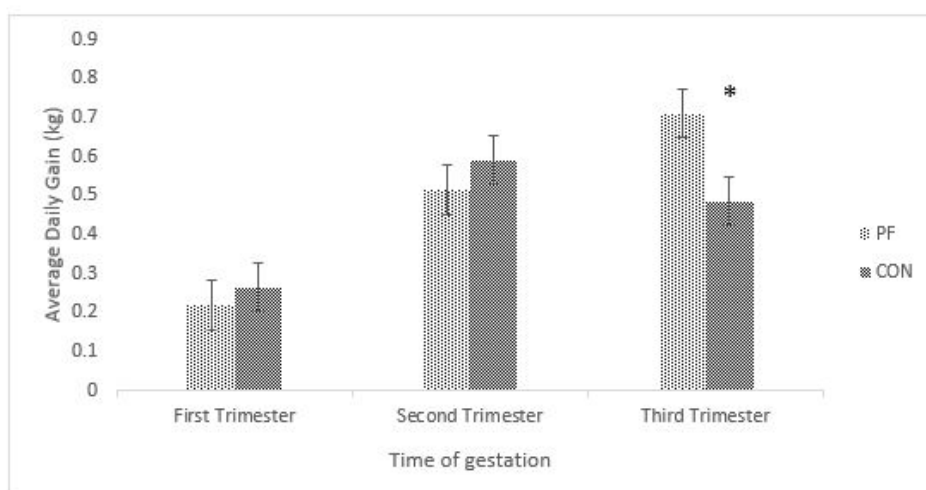
Typically, these changes in nutrient and energy requirements are not considered with current industry feeding practices, as sows tend to receive a constant allotment of feed throughout gestation and across parities, with minor adjustments in feed allowance based on a visual assessment of body condition. This can lead to an over-supply of nutrients and energy in early gestation (i.e. when requirements are low) and an under-supply of nutrients and energy in late gestation (i.e. when requirements are high; NRC, 2012). Sows in their first parity are especially sensitive to a static feeding program, as they require a greater amount of protein and energy for their own growth in addition to the growth of pregnancy-associated tissues (Moehn et al., 2011). A mismatch between the supply and demand of nutrients and energy can have negative long-term effects on sow reproductive ability (Moehn et al. 2011). The objective of this study was to determine the effects of precisely meeting estimated (daily) energy and Lysine (Lys) requirements for first parity gestating sows on sow body weight change and litter characteristics at birth.

## Methods

One hundred and seven, first parity sows were randomly assigned to a precision (PF; n=52) or control (CON; n=55) feeding program between day 2 and 9 of gestation and housed in group-pens equipped with electronic sow feeders (ESF) capable of blending two diets. The PF sows received unique daily blends of two isocaloric diets (2518 kcal/kg NE; 0.80 and 0.20% SID Lys, respectively) while the CON sows received 2.2 kg of a static blend of the diets to achieve 0.56% SID Lys. The precision blends were determined using the NRC 2012 Swine Nutrient Requirements Model to estimate daily energy and amino acid requirements and to ensure a positive energy balance (i.e. constant maternal lipid deposition of 105 g/d). Sow body weights were measured weekly, back fat thickness and loin depth were determined via ultrasound in early (~d 5), mid (~d 60), and late (~d 110) gestation. Sows were moved to farrowing crates on ~d110 of gestation and received 2 kg/d of a standard lactation diet. At farrowing, litter characteristics (e.g., number born alive, stillbirths, and birth weight) were recorded. Litters were standardized to between 10 and 12 piglets within 24 hours after birth and sows were provided ad libitum access to the lactation diet for a 21-day lactation period; at weaning sows were weighed and back fat thickness and loin depth were measured.

## Results

The PF sows had greater body weight gains in late gestation (i.e. when nutrient requirements are greatest; between day 60 and 110) versus CON sows (711 vs 486 g/d;  $P < 0.05$ ; Figure 1). The total amount of body weight gain (50.8 kg) and change in back fat thickness (1.9 mm) and loin depth (2.0 mm) between d 5 and 110 of gestation did not differ between PF and CON sows. The number of piglets born alive (11.4), stillborn (0.8), and mummified (0.2) did not differ, but PF sows had lower litter birth weights (14.6 vs 16.8 kg;  $P < 0.05$ ) and lower lactation feed intake (122 vs 107 g;  $P < 0.05$ ) than CON sows. The amount of body weight (-9.7 kg) back fat (-1.9 mm) and loin depth (-2.0 mm) loss during lactation did not differ between PF and CON sows.



**Figure 1.** Average Daily Gain (ADG) of first parity sows receiving unique daily blends of two iso-caloric diets (2518 kcal/kg NE; 0.80 and 0.20% SID Lys, respectively; precision fed; PF) or 2.2 kg of a static blend of the diets to achieve 0.56% SID Lys (CON) between day 5 and 110 of gestation.

## Conclusions

Energy, and likely amino acids, were limiting for the CON sows by late gestation as they sacrificed their own body weight gain in order to support litter growth. The long-term consequences for both the sow and her offspring remain to be elucidated. We will return these sows to their respective feeding regimens during the subsequent two parities in order to provide insight into the role of precisely matching nutrient and energy requirements during gestation on sow longevity and long-term reproductive performance. By precisely meeting estimated nutrient requirements on each day of gestation, nutrient losses to the environment will be reduced and sows may have improved longevity within the herd and produce larger litters of uniformly sized and robust piglets. Improving annual sow production by even 0.1 pig weaned (\$30/pig; value - feed cost) represents \$750,000/year for Ontario.

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1 Gohary et al., 2016. Economic value of ionophores and propylene glycol to prevent disease and treat ketosis. CVJ 57:733-740. Return based on a \$23 bolus cost and 3:1 ROI.  
2 Return based on \$0.31/kg of DM feed cost

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## **Feeding for Dual Purpose with Dual Benefit: Role of Nutrition during Pregnancy on Cow and Calf Health and Performance**

### **Alimentation à double objectif et double bénéfique : rôle de la nutrition durant la grossesse sur la santé et le rendement de la vache et du veau**

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#### **Abstract**

The periparturient and neonatal periods are the most challenging in the life cycle of dairy cattle. As a result, understanding the mechanisms coordinating metabolic and physiologic adaptations in key organs of the cow and calf during those life stages remains an active area of research. Nutritional management approaches and the environment can impact the pregnant cow and the developing calf. Ample evidence from research with non-ruminant species underscores the potential for nutrition during pregnancy to induce “developmental/nutritional programming” of the offspring, in part through “epigenetic” mechanisms that can alter gene transcription and organ function. Epigenetic changes can occur irrespective of the genetic code of the animal. Level of dietary crude protein, energy supply, heat stress, or the supply of nutrients such as methionine, choline, folic acid (i.e. “methyl donors”) during pregnancy can trigger epigenetic alterations in the offspring. Some of these factors have been studied extensively in the context of health and productivity of the peripartum cow; however, there is a paucity of information as it pertains to the calf. With the growing emphasis on nutritional management of the calf during the pre-weaning period in the context of productivity in later life, it is important to ascertain what (if any) role maternal nutrition has on the neonatal response. In the context of nutritional management to help cow and calf, recent work with rumen-protected methyl donors underscores the dual benefit of enhancing the supply of essential nutrients for the cow and calf.

#### **Résumé**

Les périodes périnatale et néonatale sont les plus exigeantes dans le cycle de vie des bovins laitiers. Ainsi donc, comprendre les mécanismes coordonnant les adaptations métaboliques et physiologiques des organes clés de la vache et du veau durant ces stades de leur vie demeure un champ de recherche actif. Les méthodes de gestion nutritionnelle et l'environnement peuvent avoir une incidence sur la vache en gestation et sur le veau en développement. Les recherches menées sur des espèces d'animaux non ruminants montrent clairement que la nutrition durant la grossesse peut induire la « programmation de la croissance/nutrition » de la progéniture, en partie à travers les mécanismes « épigénétiques » qui peuvent altérer la transcription des gènes et la fonction des organes. Des changements épigénétiques peuvent survenir, peu importe le code

génétique de l'animal. Le niveau de protéines alimentaires brutes, d'apport énergétique et de stress thermique ou l'apport en nutriments comme la méthionine, la choline et l'acide folique (c.à-d. les « donneurs méthyliques ») durant la grossesse peut déclencher des altérations épigénétiques chez la progéniture. Certains de ces facteurs ont fait l'objet d'études approfondies dans le contexte de la santé et la productivité de la vache péri-parturiente; cependant, l'information concernant le veau à cet égard est insuffisante. Alors que l'emphase augmente sur la gestion nutritionnelle du veau durant la période précédant le sevrage pour ce qui touche la productivité plus tard dans la vie, il est important de déterminer quel rôle, le cas échéant, la nutrition maternelle joue dans la réponse néonatale. En ce qui concerne la gestion nutritionnelle pour aider la vache et le veau, les récents travaux sur les donneurs méthyliques protégés contre la dégradation dans le rumen soulignent le double avantage d'améliorer l'apport en nutriments essentiels de la vache et du veau.

## **Introduction**

It is well-accepted among dairy nutritionists and physiologists that the period around parturition (“periparturient” or “transition” period) is one of the most-challenging in the life cycle of dairy cows (Lor et al., 2013; Bradford et al., 2015). Nutrient requirements of dairy cows increase as gestation progresses, largely due to the exponential growth of the gravid uterus and fetus (NRC, 2001). Conditions such as inflammation and oxidative stress and fat depot mobilization in the latter stages of pregnancy contribute to reducing voluntary dry matter intake (DMI) with a consequent shortfall in nutrient availability for the cow and fetus (Lor et al., 2013ab; Bradford et al., 2015). From an efficiency standpoint, improving nutrient utilization around the time of parturition by keeping cows healthy has been a major area of research for over 40 years (for example, Coppock et al., 1972). In particular, the reduction of DMI during transition is central to the increased risk of metabolic disorders (ketosis, fatty liver, milk fever), but also immune-related disorders that cows face. Recognition of the complexity of biological interactions occurring in the transition cow has resulted in a substantial amount of research focused on identifying mechanisms underlying metabolic, physiologic, and immune adaptations in key organs (see reviews by Lor et al., 2013ab, Roche et al., 2013; Bradford et al., 2015). Therefore, the main objectives of the present paper are to briefly summarize adaptations in metabolic events in key organs of the cow during transition, critical physiological events in the neonatal period, linkages between certain nutrients and some of the metabolic events, introduce the concept of “nutritional programming” in the context of transition cow nutrition, molecular mechanisms involved, and the potential “dual benefit” of nutritional management for cow and calf.

## **Biological Adaptations in the Transition Cow**

The focus of the dairy industry in most countries over the past several decades has been maximising milk yield/cow, thereby creating a “nutrient highway” from the daily ration and mobilization of body reserves [ $\sim 0.6$  kg fat/day,  $\sim 0.04$  kg protein/day, and  $\sim 0.15$  kg water/day during the first 8-weeks of lactation (Tamminga et al., 1997)] directly to the udder to sustain milk production. In the transition dairy cow a series of biological mechanisms bring about the prioritization of milk production at the cost of body reserves (Bauman and Currie, 1980); for example, insulin concentrations are drastically reduced and the response of hormone-sensitive lipase in adipose tissue to lipolytic stimuli in high-yielding dairy cows (e.g. low insulin, high growth hormone and catecholamines, or

high glucocorticoid concentrations) is greater, thus, facilitating lipid mobilisation and transport to tissues. The transition period is also characterized by a state of inflammation that can be measured by an increase in the production of positive acute-phase proteins (APP) such as haptoglobin and serum amyloid A (SAA), and a concomitant decrease in the production of negative APP such as albumin (Bertoni et al., 2008). At the level of the liver, the well-established triggers of these responses are the pro-inflammatory cytokines IL-6, IL-1 $\beta$ , and TNF- $\alpha$  (Bradford et al., 2015). On the other hand, oxidative stress is driven by the imbalance between the production of reactive oxygen metabolites (ROM), reactive nitrogen species (RNS), and the neutralizing capacity of antioxidant mechanisms in tissues and blood. Some of the well-established cellular antioxidants include glutathione, taurine, superoxide dismutase (SOD), and vitamins A and E (Bertoni et al., 2008). When oxidative stress overwhelms cellular antioxidant capacity, the ROM can induce an inflammatory response which is controlled via changes in mRNA abundance of transcription regulators (e.g. STAT3, NFKB). A summary of the changes in concentrations of a number of blood biomarkers associated with metabolism and health status of the cow are presented in **Table 1** (modified from Loor et al., 2013)

Most research evidence, although derived mainly from rodent work, indicates that changes in abundance of mRNA exert a major influence on physiological function. Many of the homeorhetic changes (i.e. chronic changes in multiple body tissues to support a dominant physiological state) correspond with changes in transcript abundance (i.e. abundance of mRNA) for key genes, indicating that molecular changes underpin the physiological perturbations associated with the transition from pregnancy to lactation (Loor, 2010). In the case of homeorhesis, tissue responses are coordinated by a complex network of proteins that ‘share’ information arising from cues (e.g. hormones and metabolites) from within the organ or from the external milieu (e.g. the blood). These networks have evolved so that tissues can accurately respond to external signals and either maintain homeostasis or provide priority to certain physiological functions (e.g. milk production); hence, regulation of mRNA transcription plays a pivotal role in coordinating physiological function during the transition period.

With advancing technology enabling more targeted molecular assays simultaneously across multiple tissues (Bionaz and Loor, 2012), knowledge of the mechanisms underpinning periparturient homeorhetic changes and, arguably, more importantly, the effect of environmental factors (e.g. nutrition, thermal stress) on metabolism and other physiologic functions (e.g. immune system) continues to increase (see **Table 2** for a summary). In general, it is noteworthy that changes in mRNA abundance for a number of key genes encoding important enzymes or proteins correspond with published biochemical studies of enzyme activity. With the quick development and lower costs for utilizing “high-throughput” molecular technologies, it is expected that additional mechanistic information pertaining to other levels of regulation of tissue function [e.g. non-protein coding RNA (microRNA)] will be generated in the near future.

**Table 1.** Trends in concentrations of some metabolic and inflammatory biomarkers during the periparturient period. When available the relative changes over time post-partum (days, d) are indicated. Arrows denote decrease (↓) or increase (↑) in concentration. Modified from Loor et al. (2013a).

Biomarker	Relative change between late-pregnancy and early lactation (days, d)
<b>Metabolism</b>	
NEFA	↑ to -14 d, then ↓ ~14–40 d
Glucose	↑ at 0 d, then ↓ by 7 d, then ↑ by 63 d
BHBA	↑ to 7 d, then ↓ by ~14–35 d
Urea	↑ at 0 d, then ↓ 7 d through ~14 d, then ↑ 63 d
Lactate	↑ at 0 d, then ↓ by 7 d and changes little
<b>Inflammation and liver function</b>	
Ceruloplasmin	↑ to ~4 d, then ↓ by 14 d
Haptoglobin	↑ to 4–7 d, then ↓ by ~14–18 d through 28 d
IL-6	↓ to 0 d, then ↑ by 4 d and ↓ by 9 d
TNF-α	↓ to 0 d, then ↑ to 8 d, then ↓ by 30 d
Albumin	↓ to 7 d, then ↑ by 28 d
Bilirubin	↑ to 4 d ↓ 21 d
Globulin	↓ to 0 d ↑ 63 d
Reactive oxygen metabolites	↑ to 7 d ↓ 28 d
Paraoxonase	↓ to 0 d, then ↓ to 0–7 d ↑ 63 d
Cholesterol	↓ to 0 d, then ↓ to 0–7 d ↑ 28 d
Globulin	↓ to 0 d, then ↑ to ~30 d, and changes little
Vitamin A	↓ to 0 d, then ↑ to ~30 d, and changes little
Alpha-tocopherol <sup>1</sup>	↓ to 0 d, then ↑ to ~51 d
Alkaline phosphatase	↓ to 0–28 d
Lactate dehydrogenase	↑ to 0–28 d
Gamma-glutamyl transferase	↑ to 0–28 d
Glutamic oxaloacetic acid transferase	↑ to 0–28 d
Glutathione peroxidase <sup>2</sup>	↓ to 0–51 d
Selenium	If no supplementation ↓ to 0 d, then unchanged; If supplemented from dry-off stable to 0 d, then ↑ to 21 d and remains elevated to 51 d

<sup>1</sup>Concentration of alpha-tocopherol decreases whether diet is supplemented with selenium or not. However, concentration increases to a greater extent with selenium supplementation from dry off to peak lactation. Activity of <sup>2</sup>glutathione peroxidase decreases whether diet is supplemented with selenium or not. However, supplementing selenium from dry off to peak lactation reduces the rate of the decrease in activity.

**Table 2.** Trends in key metabolic pathways in liver, adipose, and mammary tissue of dairy cattle from late-pregnancy to early post-partum. Modified from Loor (2010) and Roche et al. (2013).

Organ and pathway	Biological process	Change between late-pregnancy and	
		First week postpartum	Second to fifth week postpartum
Liver			
Ureagenesis	Arginine biosynthesis	No change to decrease	Modest increase
Glucose metabolism	Glycolysis and TCA cycle	Modest increase	No change to increase
Growth hormone signalling	IGF-1 binding/transport	Decrease	No change to decrease
Gluconeogenesis	Glucose synthesis	No change to modest increase	No change to increase
Lipoprotein metabolism	Synthesis of lipoprotein	Decrease	Decrease
Cholesterol metabolism	Synthesis and transport	No change to decrease	Modest increase
Fatty acid transport	Cellular uptake	Modest increase	Modest increase
Fatty acid oxidation	Mitochondrial and peroxisomal degradation of long-chain fatty acids	Modest increase	Modest increase
Fatty acid esterificatio	Long-chain fatty acid transfer into triacylglycerol	Increase	Increase
Ketogenesis	Synthesis of ketone bodies	Decrease	Increase
Lipid droplet formation	Desaturation and cytosolic lipid storage	Modest increase	No change to modest increase
Adipose			
Lipid and carbohydrate metabolism	Lipogenesis and adipogenesis; transcriptional regulation; glucose uptake	Marked decrease	Sustained decrease
Lipolysis	Hormone-stimulated and basal lipolysis	Modest increase	Sustained modest increase
Insulin signalling	IRS-1 phosphorylation	Decrease	Not assessed
Insulin signaling pathway	Gene expression	Decrease	Modest to moderate increase
Fatty acid transport and nutrient use	Long-chain fatty acid uptake, transport, and lactate utilisation	Modest decrease	Modest to moderate increase
Mammary			
Lipid metabolism	Fatty acid synthesis, triacylglycerol synthesis, cholesterol and sphingolipid synthesis, desaturation	Marked increase	Marked increase
Carbohydrate metabolism	Lactose synthesis	Marked increase	Marked increase
Energy metabolism	Oxidative phosphorylation and Krebs cycle	Increase	Increase
Amino acid metabolism	His, Val, Leu, and Ile metabolism	Increase	Increase

## “Functional Roles” of Nutrients: Relevance to the Transition Period

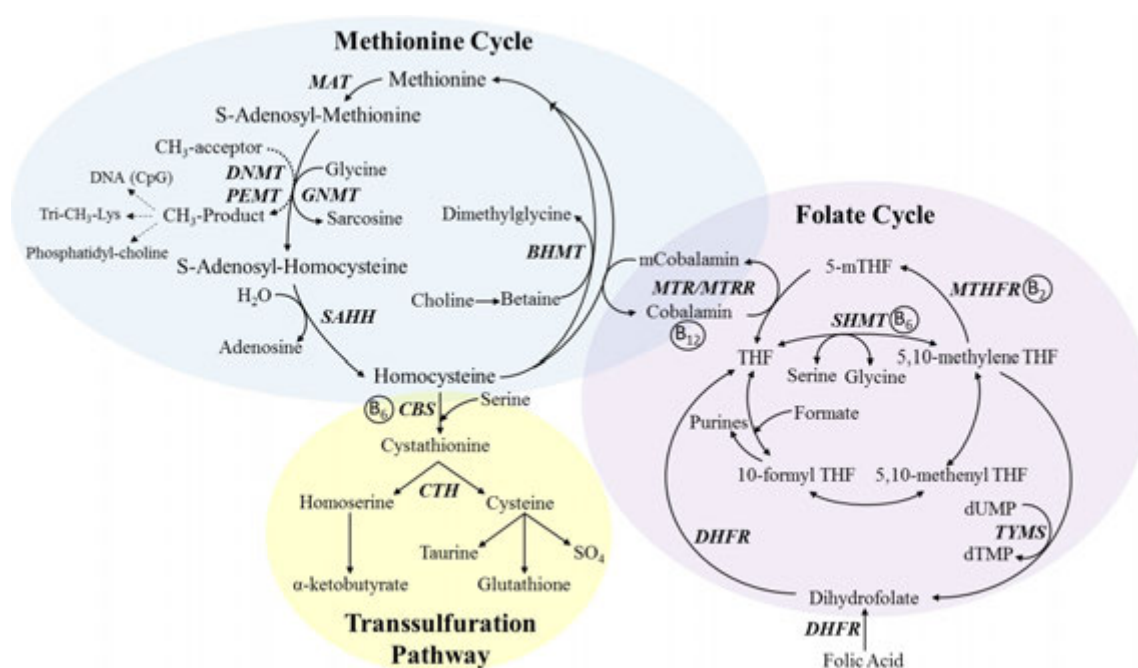
The fact that certain nutrients serve other functions besides being building blocks of macromolecules is well-established in monogastrics. Among the essential nutrients, poly-unsaturated fatty acids, essential amino acids (AA), B vitamins, choline, and trace minerals are recognized as precursors for molecules that have inflammatory activity (eicosanoids), antioxidant activity (glutathione, taurine) or serve an essential role in the catalytic activity of important enzymes (vitamin B<sub>12</sub>,

Zn, Mn). The 1-carbon metabolism pathway is particularly important in the context of functional nutrition because it links the metabolism of methionine (Met), choline, B vitamins, and folic acid through various routes that not only generate antioxidants but also carnitine (via Tri-CH<sub>3</sub>-Lysine metabolism) and phosphatidylcholine (PC), which are important in hepatic lipid metabolism (**Figure 1**). All these biological processes clearly are important in the context of dairy cattle efficiency, as exemplified, for example, by the fact that an infectious challenge in sheep (McNeil et al., 2016) or beef cattle (Burciaga-Robles, 2009) caused marked changes in tissue AA and protein metabolism. Under such conditions, AA utilization by the liver increases dramatically and diverts them from anabolic purposes such as milk protein synthesis in lactating cows or muscle deposition in growing steers or heifers. In addition, recent research has suggested that cells of the immune system and the reproductive tract have unique requirements for AA, particularly during early lactation (Jafari et al., 2006; Garcia et al., 2016; Noleto et al., 2017). The role of choline in the function of immune cells has also been recently highlighted, both in lactating cows (Garcia et al., 2018) and neonatal calves (Abdelmegeid et al., 2017). Application of the functional concept for these nutrients requires rumen-protection technology, and there already are several commercial products that can deliver AA, choline, and some B vitamins post-rationally.

The liver is the main organ where the 1-carbon metabolism operates (Speckmann et al., 2017), with PC synthesis (at least in non-ruminants) being the largest 1-carbon sink and necessary for the packaging and export of triacylglycerol into very low-density lipoproteins (VLDL; Corbin and Zeisel, 2012). At least in rodents, a deficiency in both Met and choline can induce hepatic steatosis, oxidative stress, and inflammation (Jha et al., 2014). McCarthy et al. (1968) first hypothesized that Met deficiency in ruminants may limit hepatic VLDL synthesis and be a causative factor of ketosis. Rate of hepatic VLDL synthesis was subsequently demonstrated to be lower in ruminants than monogastrics (Pullen et al., 1990). This inherent feature of ruminants is particularly important at parturition when homeorhetic adaptations lead to marked increases in blood NEFA which are taken up by liver, hence, increasing the susceptibility for hepatic lipidosis (Grummer, 1993). Several studies since then have assessed the role of Met as a potentially limiting AA in the regulation of hepatic fatty acid metabolism. Because of extensive ruminal degradation, early work evaluating Met utilized intravenous infusions or a “hydroxyl analog” of Met (Bertics and Grummer, 1999; McCarthy et al., 1968; Piepenbrink et al., 2004). The analog offers some protection against ruminal metabolism but there are currently other protection technologies that ensure a greater level of ruminal “by-pass” as well as high intestinal Met bioavailability (Berthiaume et al., 2006). A review of the literature also indicated that rumen-protected choline around parturition can reduce liver triacylglycerol concentration in some (Shahsavari et al., 2016), but not all instances (Zenobi et al., 2018).

Grummer (1993) proposed that utilization of TAG for VLDL synthesis after parturition is impaired when the level of hepatic Met is insufficient. More recent work has established an association between low levels of serum Met during the first 14 days postpartum and severe hepatic lipidosis (Shibano and Kawamura, 2006). The work of Dalbach et al. (2011) demonstrated that it is feasible to increase the serum concentration of Met during the first 2-weeks postpartum by feeding rumen-protected Met (RPM). The rate of hepatic metabolism in high-producing cows nearly doubles after parturition (Reynolds et al., 2003), which could be one reason explaining the increase in net liver uptake of Met (Larsen and Kristensen, 2013). In fact, other than histidine, Met was the

only AA for which net uptake by the liver increased between pre and postpartum (Larsen and Kristensen, 2013). Aspects of Met metabolism in liver via the 1-carbon metabolism (Figure 1) are well-described in monogastrics, and to some extent in classical studies with sheep (Snoswell and Xue, 1987). In a recent study from our group, Zhou et al. (2017) provided the first demonstration that activity of betaine-homocysteine methyltransferase (BHMT), methionine synthase (MTR), and cystathionine synthase (CBS) (Figure 1) increases around parturition and might be responsive to the supply of Met.



**Figure 1.** 1-carbon metabolic pathways. Enzymes (in bold): BHMT = betaine homocysteine methyltransferase; CBS = cystathionine beta synthase; CTH = cystathionine gamma-lyase; DHFR = dihydrofolate reductase; DNMT = DNA methyltransferase; GNMT = glycine N-methyltransferase; MAT = methionine adenosyltransferase; MTHFR = methylenetetrahydrofolate reductase; MTR = 5-methyltetrahydrofolate-homocysteine methyltransferase; MTRR = 5-methyltetrahydrofolate-homocysteine methyltransferase reductase; PEMT = phosphatidylethanolamine N-methyltransferase; SAHH = s-adenosyl-homocysteine hydrolase; SHMT = serine hydroxymethyltransferase; TYMS = thymidylate synthetase. Substrates: 5-mTHF = 5-methyl-tetrahydrofolate; dUMP = deoxyuridine monophosphate; dTMP = thymidine monophosphate; THF = tetrahydrofolate. B vitamins: B<sub>2</sub> = riboflavin; B<sub>6</sub> = Pyridoxal 5'-phosphate; B<sub>12</sub> = cobalamin.

## Rumen-protected Methionine in Transition Cows

Increasing the delivery of Met to the liver via supplementation of rumen-protected sources is particularly important for the animal, not only because of the key role of Met in milk protein synthesis but also for production of glutathione and taurine [intracellular antioxidants, Figure 1. (Atmaca, 2004)], and provision of methyl groups (Finkelstein, 1990). Thus, although not the only essential nutrient with such a role, Met is one example of an AA with a clear functional role (Figure 1). A series of recently published studies with dairy cows has demonstrated the unique role of Met, beyond serving as a source of AA for protein synthesis, in the context of allowing cows to maintain more consistent rates of dry matter intake around parturition, reduce inflammation and oxidative stress, have a better innate immune function, remain healthier, and optimize production of milk (Osorio et al., 2013a; Zhou et al., 2016a; Batistel et al., 2017b, 2018).

Supplementation of Met during the periparturient period concomitantly increases milk yield, milk protein, and milk fat soon after calving (Ordway et al., 2009; Osorio et al., 2013a). These responses are in large part driven by enhancing Met availability and by additional flux of Met through the Met cycle in liver, which consequently increases the production of downstream compounds such as cysteine (Cys). Just as Met, Cys is a sulfur-containing AA and both contribute sulfur bonds during milk protein synthesis in the mammary gland (Pocius et al., 1981). In terms of milk production, work in our lab feeding RPM has detected positive responses in terms of maintaining consistent rates of DMI prepartum (last 21 days) and faster and greater rates of DMI during the first 30 to 60 days after calving (**Table 3**). The milk production responses have been consistent with research from other groups demonstrating benefits of postpartum supplementation of RPM (St-Pierre and Sylvester, 2005).

**Table 3.** Summary of major production responses in cows fed a rumen-protected Met supplemented diet during the transition period and early lactation.

Item	Experiment		
	Osorio et al. (2013a)	Zhou et al. (2016a)	Batistel et al. (2017b)
Diet crude protein, %	15 (pre), 17.5 (post)	14.6 (pre), 17.3 (post)	15.7 (pre), 17.4 (post)
Lys, % of metabolizable protein (MP)	6.1-6.6	6.2-6.6	6.3-6.5
Lys, grams in MP	81 (pre), 112 (post)	85 (pre), 148 (post)	89 (pre), 156 (post)
Met, % of MP	2.15-2.35 (1.8-1.9) <sup>2</sup>	2.3-2.4 (1.8-1.9) <sup>2</sup>	2.2-2.3 (1.7-1.8) <sup>2</sup>
Met, grams in MP	29 (pre), 40 (post)	33 (pre), 55 (post)	32 (pre), 56 (post)
Average response relative to the control diet <sup>1</sup>			
DMI, kg/d			
Prepartum	+0.5	+1.1	+1.2
Postpartum	+2.1	+2.0	+1.6
Milk yield, kg/d	+3.4	+3.8	+4.3
Energy-corrected milk, kg/d	+3.9	+4.1	+4.6

<sup>1</sup>Corn silage-based diet. Rumen-protected Met was supplemented from -21 through 30 days in milk in Osorio et al. (2013a) and Zhou et al. (2016a) or until 60 days in milk in Batistel et al. (2017b). <sup>2</sup>Range in the control unsupplemented diet. <sup>3</sup>Amounts in the prepartum (pre) or postpartum (post) diet.

The transient inflammatory-like status around parturition appears to be a “normal” aspect of the adaptations to lactation (Bradford et al., 2015), with its positive or negative impact depending on its degree. Cows that approach parturition with a greater (but still subclinical) level of circulating cytokines have greater inflammation and oxidative stress, and lower liver function, often through 30 days in milk, along with lower milk yield and lower postpartum DMI (Bertoni et al., 2008). In addition to their fundamental function in immunity, cytokines (ILs), interferons (IFNs) and TNF- $\alpha$  also elicit pathophysiological effects. This leads to what is commonly known as “sickness behavior”, whose primary manifestation is satiety. Similar to how cows react during an inflammatory state around parturition, the reduction in DMI around calving is an example of this behavior. In mice, these cytokines have been shown to reduce meal size and duration, as well as decrease meal frequency and prolong inter-meal intervals (Plata-Salaman, 1995). Furthermore, cytokines directly affect the hypothalamus. IL-1 $\beta$  and IFN act directly and specifically on the glucose-sensitive neurons in the brain “satiety” and “hunger” sites (Plata-Salaman, 1995). Thus, the increased DMI observed when feeding RPM can be partly explained by a reduction in inflammation, as it directly (at the hepatic level and by dampening the immune cell overresponse) and indirectly (reducing oxidative stress) decreases circulating pro-inflammatory cytokines. In our studies with supplementation of RPM, we have detected consistent responses in a number of biomarkers in plasma and liver tissue, indicating that Met helps reduce inflammatory and oxidative stress status of the cows

In research from our group and others with RPM supplementation during the periparturient period we have consistently detected improvements in immunometabolic status (Osorio et al., 2014ab; Sun et al., 2016; Zhou et al., 2016a; Batistel et al., 2018). Furthermore, compared with rumen-protected choline, greater supply of Met resulted in increased antioxidant concentration in liver tissue despite a lower concentration of PC (Zhou et al., 2017). Those responses were due to the greater abundance of phosphatidylethanolamine methyltransferase and CBS (Zhou et al., 2017). A greater supply of choline did not change the mRNA abundance of BHMT and MTR in cows with a greater supply of choline. A summary of major effects of RPM on immunometabolic biomarkers is in **Table 4**.

**Table 4.** Summary of additional beneficial effects of feeding rumen-protected methionine during the transition period and early lactation. ↑ = beneficial increase; ↓ = beneficial decrease; ↔ = no change in concentration.

<b>Biomarker</b>	<b>Response<sup>1</sup></b>	<b>Biological function</b>
<b><i>Metabolism</i></b>		
Carnitine	↑ (liver)	β-oxidation of Fatty Acids
Cholesterol	↑↑ (plasma)	Lipoprotein metabolism
<b><i>Inflammation</i></b>		
IL-1beta	↓ (plasma)	Pro-inflammatory cytokin
Haptoglobin	↓↓ (plasma)	Inflammation signa
Albumin	↑↑ (plasma)	Acute-phase response
<b><i>Oxidative stress</i></b>		
Reactive oxygen metabolites (ROM)	↔/↓ (plasma)	Peroxides, superoxide, OH-radicals
Glutathione	↑↑ (liver, blood)	Antioxidant
Taurine	↔/↑ (plasma)	Antioxidant
Antioxidant capacity	↔/↑ (plasma)	Total antioxidants in blood
Paraoxonase	↑↑ (plasma)	Antioxidant enzyme

<sup>1</sup>Relative to a control or rumen-protected choline supplemented diet (Osorio et al., 2013a; Zhou et al., 2017; Sun et al., 2016; Batistel et al., 2018).

## Maternal Nutrition and the Developing Calf

### *The Concept of Nutritional Programming*

The concept that differences in nutritional experiences at critical periods in early life, both pre- and postnatally, can program an individual's development, metabolism and health for the future is generally referred to as “nutritional programming” (for example, Wu et al., 2004). There is ample evidence in non-ruminant species that maternal dietary methyl donors, such as Met, choline, folic acid, and betaine, play a role in nutritional programming (for example, Ji et al., 2015). These nutrients can elicit a programming effect, partly through “epigenetics”, i.e. phenotypic changes that do not involve alterations in the DNA sequence. Epigenetic changes can occur through methylation of DNA, RNA, and histones. Dietary methyl donors serve as precursors of S-adenosyl-methionine (SAM, Figure 1) that could be used via methyltransferases (for example, GNMT) to methylate DNA, RNA and histones (Hollenbeck, 2012; Lin et al., 2014). In newborn piglets, it was demonstrated that maternal folic acid supplementation altered the expression of genes associated with immunity, oxidative stress response and hepatic energy metabolism (Liu et al., 2013). In addition, supplementing betaine to sows during pregnancy resulted in alterations in the expression of gluconeogenic genes in the liver of newborn piglets, partly through changes in DNA methylation (Cai et al., 2014).

Other epigenetic changes unrelated to methylation involve acetylation of histones and/or changes in abundance of microRNA (non-protein coding RNA). Alterations in microRNA abundance (at least in non-ruminants) are particularly important for fine-tuning regulation of several cellular

process (Aguilera et al., 2010) that modulate innate immune function, including regulation of senescence, differentiation, adherence capacity and cytokine production (Gantier, 2013). These observations could be of interest if linked to the fact that epigenetic marks are candidates for bearing the memory of specific intrauterine nutritional exposures causing alterations in long-term programming of mRNA abundance, and consequently inducing developmental adaptations in physiology and metabolism. Although the exact mechanisms whereby mature microRNA can repress or activate translational activity (Morales et al., 2017), promote destabilization of target mRNA, and regulate the abundance level of target genes remains under debate (Eulalio et al., 2008), their biological role seems unequivocal. To add more complexity, it is now understood that epigenetic regulation of histones (i.e. methylation or acetylation) also can impact microRNA abundance (Morales et al., 2017). We have recently demonstrated alterations in a number of microRNA that are expressed in adipose tissue during the periparturient period (**Table 5**). Both, BCS at calving and prepartum overfeeding can alter the abundance of certain microRNA involved in inflammation and fat deposition (e.g. miR-99a, miR-145, miR-155).

**Table 5.** Details and functions of microRNA expressed in adipose tissue of periparturient cows (Vailati-Riboni et al., 2016).

microRNA	Function or expression pattern
<b><i>Infiltration of immune cells</i></b>	
miR-26b	Abundance is associated with the number of macrophages infiltrating the fat depo Affected by levels of circulating TNF- $\alpha$ , leptin and resistin
miR-126	Directly inhibits <i>CCL2</i> mRNA abundance
miR-132	Abundance is associated with the number of macrophages infiltrating fat depots Activates inflammation via NF $\kappa$ B signalling and the transcription of <i>IL8</i> and <i>CCL2</i> Lower abundance is associated with increased secretion of IL-6
miR-155	Abundance is associated with the number of macrophages infiltrating fat depot
miR-193	Indirectly inhibits <i>CCL2</i> abundance through a network of transcription factors
<b><i>Inflammation and lipolysis</i></b>	
miR-99a	Negative correlation with secretion of IL-6 and level of free fatty acids
miR-145	Affects secretion of TNF- $\alpha$ , regulating lipolysis
miR-221	Lower abundance is associated with high levels of TNF- $\alpha$
<b><i>Proadipogenic</i></b>	
miR-103	Regulates mRNA abundance of <i>PPARG</i> , <i>PANK1</i> , <i>CAV1</i> , <i>FASN</i> , <i>ADIPOQ</i> and <i>FABP4</i>
miR-143	Regulates mRNA abundance of <i>ERK5</i> , <i>SLC2A4</i> , <i>TFAP2A</i> , <i>LIPE</i> , <i>PPARG</i> , <i>CEBPA</i> , and <i>FABP4</i>
miR-378	Targets <i>PPARG</i> mRNA abundance through the MAPK1 pathway

## Nutritional Programming in Ruminants

The available literature on nutritional programming of various physiological aspects specifically

in ruminants (for example, embryo development, placental function, muscle and fat deposition) has been reviewed in at least 3 recent comprehensive papers (Sinclair et al., 2013, 2016; Chavatte-Palmer et al., 2018). For the purpose of the present short-review we list in **Table 6** nutritional factors relevant to ruminants for which there are known epigenetics roles. We also list available information on programming effects of body composition and appetite. Most available data come from sheep and beef cattle, and there is some evidence that mammary development, fertility, welfare and behavior, and immune function might be susceptible to nutritional programming (Sinclair et al., 2016).

**Table 6.** Dietary/nutritional factors and known epigenetic effects.

Nutrient category	Nutritional factor	Epigenetic mechanism
Fatty acids	Butyrate (from gastrointestinal fermentation)	Histone modification
One-carbon metabolism	Methionine, choline, betaine, folic acid, arginine, Vitamins B <sub>2</sub> , B <sub>6</sub> , and B <sub>12</sub>	DNA methylation
Polyphenols	Genistein, quercetin, resveratrol	microRNA, histone modification
Minerals	Cu, Zn, Co, Ni	microRNA, histone modification
Dietary changes	Level of dietary energy	microRNA
	Methyl donor deficiency	DNA methylation, histone modification
	Protein restriction	DNA methylation, histone modification
Programming event	Biological response	
Skeletal muscle	Muscle fiber formation, mass or size	DNA methylation, microRNA
Fat	Increased deposition	DNA methylation
Appetite regulation	Alteration of neural circuits (hypothalamus)	DNA methylation
	Alterations in endocrine factors	Orexigenic gene upregulation
		Anorexigenic gene downregulation

## Utero-placental Nutrient Transport and Metabolism

In dairy cows, the final trimester of gestation is characterized by marked fetal growth, and proper placental transfer of nutrients is required to ensure adequate fetal development (NRC, 2001). Besides maternal nutrient availability, it is well-established in non-ruminants that expression and activity of specific transporters in the placenta influence the transport of nutrients from maternal to fetal circulation (Jones et al., 2007). The anatomical structure of the placenta precludes direct contact of maternal and fetal blood, emphasizing the importance of protein transporters, concentration gradients and diffusion channels for membrane nutrient exchange (Brett et al., 2014).

In bovine, the areas of maternal-fetal interface are limited to discrete round structures named placentomes, which consist of maternal caruncles interdigitating with fetal cotyledons (Brett et al., 2014; Bridger et al., 2007). In order to reach the fetal blood, nutrients from maternal circulation must surpass the syncytiotrophoblasts (Firth, 1966), which contain two polarized membranes: the

microvillus membrane facing maternal circulation and the basal plasma membrane facing the fetal vascular structure (Brett et al., 2014). Both membranes have low permeability, hence, constituting rate-limiting steps for the transport of medium and large substrates into fetal circulation (Brett et al., 2014). Consequently, nutrients are predominantly absorbed and translocated into fetal circulation by nutrient-specific transport proteins situated within the microvillous membrane and basal plasma membrane (Lager and Powell, 2012).

Besides performing an indispensable function delivering essential nutrients for fetal growth, placental tissue (as other mammalian cells) has an array of additional nutrient-sensing signaling pathways, such as the mammalian target of rapamycin (mTOR) complex (Jansson et al., 2013). For instance, in humans, mTOR alters the activity of placental AA transporters in response to the level of nutrient supply (Roos et al., 2009). These nutrient-signaling mechanisms are also sensitive to hormones such as insulin, thus, shifts in the endocrine environment resulting from alterations in dietary energy density could alter nutrient delivery to the placenta (and fetus), as well as the abundance of various nutrient transporters. A recent study from our laboratory provided some of the first data demonstrating the presence of various nutrient transporters in term placenta from dairy cows (**Table 7**). These data are important in the context of understanding potential linkages between dietary nutrient supply and availability to the fetus.

**Table 7.** Transporters of amino acids, fatty acids, glucose and vitamins detected in term placentome from Holstein cows (Batistel et al., 2017a).

Transporter and gene symbol	Transporter and gene symbol
Glutamate (SLC1A1)	Glucose, galactose, mannose (SLC2A1)
Neutral AA (SLC1A5)	Glucose, galactose, mannose, maltose (SLC2A3)
Heavy chain AA (SLC3A2)	Glucose/fructose (SLC2A4)
Taurine transporter (SLC6A6)	Glucose (SLC2A5)
Branched-chain and aromatic AA (SLC7A5)	Glucose (SLC2A6)
Branched-chain and aromatic AA (SLC7A8)	Glucose and fructose (SLC2A8)
Neutral AA (SLC38A1)	Glucose and galactose (SLC2A9)
Neutral AA (SLC38A2)	Glucose (SLC2A10)
Sodium-dependent AA (SLC38A6)	Glucose (SLC2A11)
Glu, Gln, His, Ser, Ala, Asn (SLC38A7)	Glucose (SLC2A12)
Sodium-dependent AA, Ala, GLn, Glu, Asp (SLC38A10)	Glucose cotransporter (SLC2A13)
Sodium-dependent AA (SLC38A11)	Glucose, galactose, mannose (SLC5A11)
Sodium-independent, Leu, Phe, Val, Met (SLC43A2)	Multivitamin (SLC5A6)
Long chain fatty acid (SLC27A1)	Betaine (SLC6A12)
Long chain fatty acid (SLC27A2)	Thiamine (SLC19A2)
Long chain fatty acid (SLC27A3)	Thiamine (SLC19A3)
Choline (SLC44A1)	
Choline (SLC44A3)	
Folate (SLC46A1)	

## Maternal Prepartum Nutrition and Dairy Calf Development

### *General*

Modern feeding systems for dairy cattle recommend sufficient feed in late-pregnancy (i.e. last trimester of gestation) to provide ~1.5 times maintenance energy requirements to maintain maternal body condition and fetal growth (Quigley and Drewry, 1998). There are comprehensive reviews published on the potential for maternal plane of nutrition (for example, Quigley and Drewry, 1998; Funston and Summers, 2013; Khanal and Nielsen, 2017) or micronutrient supply (Reynolds et al., 2010; Bach, 2012) to alter fetal and neonatal growth of cattle and sheep. The consensus seems to be that, unless nutrition is markedly inadequate (e.g. feed deprivation, nutrient imbalances, poor quality forage in the early dry period), prepartum nutrition should not markedly affect fetal development or chemical composition. Although the current approach for feeding high-producing dairy cows during the last 3-4 weeks prepartum is designed to provide additional energy to support fetal growth (and adapt the rumen to a lactation diet), the specific requirements for essential nutrients (AA, trace minerals, B vitamins, choline) by the cow, conceptus and fetus remain unknown. However, there is evidence from bovine studies that a limitation in the supply of methyl donors can impact not only physiological responses of the cows, but also important developmental aspects in the calf that could have long-term consequences (discussed below).

### *Effects of Maternal Dietary Energy Level on Calf Development*

Although there are few published studies in dairy cows designed to address the relationship between prepartal energy intake and calf development, there is some evidence that over- and under-feeding energy can have an effect. For example, cows fed a lower-energy diet (1.25 vs. 1.55 Mcal/kg dry matter; ~13% crude protein) for the last 21 days prior to parturition delivered calves that were lighter (39.2 vs. 43.9 kg), shorter (74.7 vs. 78.0 cm), and had lower body length (72.6 vs. 74.2 cm) (Gao et al., 2012). In contrast, feeding a higher-energy diet (1.47 vs. 1.24 Mcal/kg; ~15% crude protein) for the last 3 weeks prepartum resulted in lighter calves (44.0 vs. 48.6 kg) at birth (Osorio et al., 2013b). In cattle, fetal skeletal muscle matures during late-gestation, hence, prenatal plane of nutrition of the cow at this time would impact muscle growth of the calf (Sinclair et al., 2016). Such response explains in part the lower body weight at birth and muscle mass when dams are nutrient-restricted during gestation. Despite this evidence, it should be noted that a number of studies in which the energy density (or source of energy) of the prepartum diet has been modified to address metabolic responses of the cow did not report differences in calf birth weight (for example, Rabelo et al., 2003; Dann et al., 2005, 2006; Guo et al., 2007; Janovick and Drackley, 2010). Although it is challenging to draw major conclusions across the available published experiments with dairy cows (for example, confounding effects of environment, diet composition), data from pigs (for example, folic acid and betaine) underscores the potential for specific nutrients in the pregnant cow diet to potentially alter calf development. Hence, variations in micronutrient availability to the cow and calf in the published studies dealing with the role of dietary “energy density” might account for lack of consistency in terms of measurable effects on the calf, particularly birth body weight. Without longitudinal performance data for the calves it would be impossible to determine programming effects that might be induced by maternal energy nutrition.

## Programming of Neonatal Calf Immune Function

### *Maternal Plane of Nutrition, Body Condition Score, and Metabolic Stress*

Plane of dietary energy prepartum altered the profiles of T lymphocytes in neonatal calves (Gao et al., 2012). As discussed in a previous section, the lower energy diet prepartum led to lower calf birth weight, but also a lower ratio of CD4<sup>+</sup> to CD8<sup>+</sup> T cells, suggesting those calves were less “immunocompetent”. Additional support for a negative effect on immune function was the lower concentration of interleukin-4 (IL-4), a key cytokine involved in the synthesis of immunoglobulins and also the profile of CD4<sup>+</sup> cells, which would be important in conferring the young calf immune protection against pathogens. Other aspects of immune function such as the oxidant status of the calves were altered, including lower total antioxidant capacity and superoxide dismutase activity, while activity of glutathione peroxidase and concentrations of lipid peroxide products were increased. Although this study did not address epigenetic mechanisms per se, data generated by our group indicated that maternal dietary energy could alter neonatal calf mRNA abundance of subsets of genes with important roles in the innate immune response (by neutrophils) including the control of cytokine production (Osorio et al., 2013b). These molecular effects were not associated with differences in colostrum quality.

The use of body condition score (BCS) to assess body fat and muscle reserves is well-established (for example, Roche et al., 2013), with low values reflecting emaciation and high values obesity. The BCS at which a cow calves, her nadir BCS, and the amount of BCS lost after calving are associated with milk production, reproduction, and health. Furthermore, data from our laboratory has demonstrated associations between BCS at calving and altered profiles of systemic (plasma) and molecular (liver, adipose) biomarkers of inflammation, oxidative stress, and metabolism (for example, Akbar et al., 2015). For instance, compared with a calving BCS of 3.0-3.25, calving at BCS greater or lower than that not only increases risk of metabolic disorders (for example, fatty liver and ketosis), but could also trigger a longer period of inflammation and oxidative stress. Hence, besides body reserves, BCS at calving represents an evaluation of “stress status” in dairy cows. The use of an “oxidant status index” (OSi) has recently been proposed as way to determine stress status of pregnant cows in late-pregnancy (Ling et al., 2018). A priori, the OSi (based on plasma NEFA, haptoglobin, ROS, RNS) seems to capture metabolic stress regardless of BCS; however, higher plasma concentrations of NEFA and haptoglobin during the last 28 days prior to calving also were associated with degree of metabolic stress regardless of BCS (Ling et al., 2018).

The fact that exposure to stresses (for example, heat stress) during late-pregnancy causes impaired immune function (Tao et al., 2012), milk and reproductive performance (Monteiro et al., 2016), and mammary development (Skibieli et al., 2018) in offspring has highlighted the importance of evaluating calf development in the context of suitable biomarkers of stress. In a recent study classifying cows according to OSi in the last 28 days prior to calving, it was reported that calves born to cows classified as experiencing higher metabolic stress had lower body weight at birth and throughout the first 4 weeks of age (Ling et al., 2018). Those calves also had greater ROS and RNS concentrations, along with greater inflammatory biomarkers (plasma haptoglobin, TNF- $\alpha$ ), indicating greater basal inflammatory status. In contrast, lipopolysaccharide (LPS)-induced inflammatory responses were less robust in calves exposed to higher maternal biomarkers of metabolic stress, suggesting a compromised immune response. Whether these negative maternal effects of metabolic stress were caused via epigenetics mechanisms is unknown.

## ***Maternal Dietary Micronutrients***

Nutritional supplementation with vitamins, minerals, and other micronutrients during pregnancy have been intensively studied as effectors for immune system activation, not only for the cow, to face the transition period, but also for the offspring to adapt to the extra-uterine life (Girard et al., 1995; Thornton, 2010). In the context of B vitamins and folic acid, and although requirements in dairy cows and calves are unknown, it is commonly thought that synthesis of those compounds by ruminal microorganisms is sufficient to meet demands of cow and fetus (for example, Girard et al., 1995; Ragaller et al., 2009). The absence of differences in birth body weight when folic acid supply increased during late-pregnancy is opposite to data from non-ruminants (Girard et al., 1995), underscoring the fact that dairy cows fed typical diets during late-pregnancy are in “adequate” folic acid status. Trace mineral elements such as Cu, Cr and Zn have important roles in the health and immunity of periparturient dairy cows (Spears and Weiss, 2008). The implications of trace mineral deficiency or impaired placental transfer of these minerals to fetal and neonatal ruminant metabolism have been studied for more than 30 years (Hidiroglou, 1980). For instance, dairy calves supplemented with an injectable trace mineral complex containing Se, Cu, Zn, and Mn increased PMN and glutathione peroxidase activity, while reducing incidence of diarrhea, pneumonia, and otitis (Teixeira et al., 2014). These constitute an example of the innate immune response of the animal, one in which cells such as PMN are partly regulated via signaling pathways and changes in mRNA expression.

Although changes in mRNA abundance are known to partly control adaptations in PMN due to inflammation, more recent studies have concluded that epigenetic modifications through the activity of microRNA are an important part of the regulation of several cellular processes (Aguilera et al., 2010) that modulate PMN function, including regulation of senescence, differentiation, adherence capacity, and cytokine production (Gantier, 2013). These observations and the fact that epigenetic markers are candidates for bearing the memory of specific intrauterine nutritional exposures, causing alterations in long-term mRNA abundance and cell function, led us to study the potential role of maternal organic trace mineral supplementation during late-pregnancy on microRNA profiles in the PMN from neonatal calves (Jacometo et al., 2015). Despite the lack of effect of organic (ORG) vs. inorganic (INO) trace mineral supplementation on calf birth weight, the abundance of toll-like receptor pathway genes indicated a pro-inflammatory state in INO calves, with greater abundance of various inflammatory mediators. The lower abundance of miR-155 and miR-125b in ORG calves indicated the potential for maternal ORG trace minerals in regulating the PMN inflammatory response, at least via alterations in mRNA and microRNA abundance. Because these data were from “basal” non-stimulated cells, further studies would be helpful in evaluating the benefit of these responses under challenged conditions, e.g. after an inflammatory or pathogen challenge.

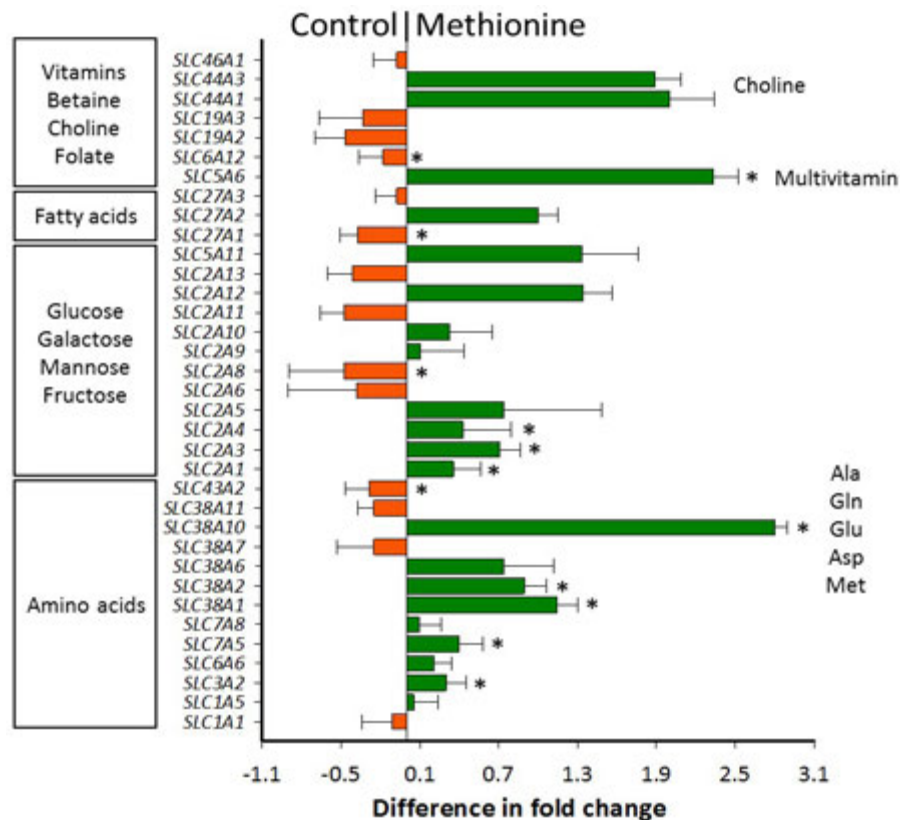
## ***Maternal Dietary Methyl Donors***

### ***Effects on the Placenta***

Fetal growth is greatly increased during the final third trimester of gestation, and proper placental transfer of nutrients is required to ensure adequate development (Borowicz et al., 2007). Besides blood flow, the expression and activity of specific transporters in the placenta, e.g. glucose, AA,

choline can limit nutrient delivery to the fetus (Jones et al., 2007; Regnault et al., 2005). Although not all micronutrients have been studied in the context of maternal transport mechanisms, it is well-established in non-ruminants that placental AA transport is dependent on maternal circulating AA profiles and transport capacity. In fact, both of these are affected by the composition and amount of AA in the diet (Brown et al., 2011). Among the essential AA, the gradual decrease in plasma Met concentration between -21 and 10 d relative to parturition in dairy cows suggested that it could be limiting not only for the cow but also the calf during the last stages prior to calving (Zhou et al., 2016b). A classical study demonstrated the essentiality of Met for normal embryo development (Coelho et al., 1989), i.e. embryos cultured in medium containing serum from cows supplemented with RPM, which increased serum Met from 4.8 to ~75 µg/mL after 14 days of supplementation, developed normally in vitro compared with embryos cultured in serum from cows not receiving supplemental RPM (Coelho et al., 1989). Thus, in the context of maternal nutrition and fetal development, our lab has placed special interest on the link between supply of Met and choline. As such, we have begun to study not only responses to changes in the post-ruminal supply of these nutrients at the cow and calf level, but also in terms of placental metabolism.

In two recent studies, we have reported associations between supplemental RPM during the last 3 weeks prior to calving and molecular mechanisms in placenta that may account for greater calf birth body weight (Batistel et al., 2017a, 2019). In **Figure 2**, we depict the alterations in mRNA abundance of nutrient transporters in response to supplemental RPM. Clearly, enhancing the supply of Met in late-pregnancy can upregulate abundance of AA, glucose, and vitamin transporters, potentially enhancing the availability of those nutrients to the developing fetus. We have speculated that these responses at the placental level are partly due to the greater DMI in cows fed RPM (see Table 2).



**Figure 2.** Fold-change difference (Methionine vs. control; negative values denote downregulation in mRNA abundance and positive values upregulation) of nutrient transporters reported in Table 4. Cows were fed a basal control diet or the basal diet plus ethyl-cellulose rumen-protected methionine (0.09% of diet dry matter during the last 28 days of pregnancy (Batistel et al., 2016).

There is evidence in non-ruminants that nutrients or dietary changes can trigger changes in epigenetic marks in placenta, with some of those changes being associated with the sex of the fetus. For example, high-fat diets triggered sex-specific gene expression in mouse placenta (Gallou-Kaban et al., 2010; Gabory et al., 2012). Those data led us to explore whether the enhanced supply of methyl donors in late-pregnancy could also alter epigenetic (for example, DNA methylation) marks in the placenta in relation to calf sex (Batistel et al., 2019). We not only evaluated total DNA methylation but also used metabolomics and gene expression analyses to assess metabolism through the 1-carbon metabolism pathway. Selected results are summarized in **Table 8**. A major finding from that analysis was that 1-carbon metabolism was affected differently by Met supply in placenta from cows delivering female compared with male calves. For instance, compared with placenta from male Controls, male Met placenta had greater concentrations of glutathione (derived from transsulfuration, Figure 1) and also greater MTR activity. In contrast, compared with Control, concentrations of Met and SAM, DNA methylation, and mRNA abundance of DNA methyltransferases was greater in placenta from cows fed Met and carrying female calves. The

lower global DNA methylation and the greater abundance of *DNMT3A* and *DNMT3B* in placenta from cows carrying female calves and receiving a greater supply of Met underscored the complex interaction between metabolism and epigenetic modifications. A possible explanation for these seemingly-opposite results is that the extra energy available to the placenta due to greater DMI in cows carrying female calves and fed Met stimulated active DNA methylation (Bochtler et al., 2017) which was followed by *de novo* methylation (DNMT3A and DNMT3B). The DNA methylation changes detected may be one of the mechanisms behind the differences in calf growth through 9 weeks of age (discussed below).

**Table 8.** Selected metabolite concentrations, enzyme activity, DNA methylation, and mRNA abundance of DNA methyl transferases in term placenta from Holstein cows fed a basal control diet or the basal diet plus ethyl-cellulose rumen-protected methionine (0.09% of diet dry matter during the last 28 days of pregnancy (Batistel et al., 2016). <sup>ab</sup>Means differ ( $P < 0.10$ ) between control and methionine groups within calf sex.

Items measured in placenta	Maternal dietary groups			
	Male calves		Female calves	
	Control	Methionine	Control	Methionine
Metabolites (relative units)				
Methionine	$646 \times 10^3$	$663 \times 10^3$	$627 \times 10^{3b}$	$711 \times 10^{3a}$
S-Adenosyl-methionine (SAM)	$123 \times 10^3$	$159 \times 10^3$	$123 \times 10^{3b}$	$190 \times 10^{3a}$
Folic acid	97	89	76	109
Betaine	$32 \times 10^3$	$33 \times 10^3$	$31 \times 10^3$	$35 \times 10^3$
Choline	$355 \times 10^3$	$391 \times 10^3$	$364 \times 10^3$	$382 \times 10^3$
Cysteine	$13 \times 10^3$	$15 \times 10^3$	$13 \times 10^3$	$14 \times 10^3$
Taurine	$31 \times 10^3$	$31 \times 10^3$	$31 \times 10^3$	$30 \times 10^3$
Glutathione	$190 \times 10^{3b}$	$383 \times 10^{3a}$	$206 \times 10^3$	$267 \times 10^3$
Vitamin B <sub>12</sub>	35 <sup>b</sup>	66 <sup>a</sup>	26	24
Enzyme activity (nmol/h/mg protein)				
BHMT	4.0	8.2	4.0	7.8
MTR	8.1 <sup>b</sup>	13.2 <sup>a</sup>	8.6	9.8
CBS	6.6	8.7	7.1	8.9
Global DNA methylation (%)	3.2	3.5	4.6 <sup>a</sup>	3.2 <sup>b</sup>
<i>DNMT3A</i> mRNA	0.63	0.59	0.48 <sup>b</sup>	0.61 <sup>a</sup>
<i>DNMT3B</i> mRNA	0.41	0.40	0.59 <sup>b</sup>	0.72 <sup>a</sup>

## Effects on Calf Development in Utero and the First Weeks of Life

The recent studies of Batistel et al. (2017b, 2018) not only confirmed the benefits of enhancing the supply of Met to dairy cows during the periparturient period on DMI, production, and health but also underscored the responsiveness of the placenta. More importantly, they demonstrated that calf development in utero and growth during the first 9 weeks of life responded to an increase in maternal supply of Met (Alharthi et al., 2018). That study allowed us to also look at a potential interaction between colostrum and maternal supply of Met (**Table 9**). A total of 39 calves were in Control ( $n = 22$  bulls, 17 heifers) and 42 in Met ( $n = 20$  bulls, 22 heifers). At birth, calves were randomly allocated considering dam treatment and colostrum as follows: 1) calves from Control cows and colostrum from Control cows ( $n = 21$ ); 2) calves from Control cows and colostrum from Met cows ( $n = 18$ ); 3) calves from Met cows and colostrum from Met cows ( $n = 22$ ); and 4) calves from Met cows and colostrum from Control cows ( $n = 20$ ). All calves were housed, managed, and fed individually during the first 9 weeks of life.

Despite greater daily DMI pre-partum in cows fed Met (15.7 vs.  $14.4 \pm 0.12$  kg/d), colostrum quality and quantity were not affected by maternal diet. At birth, Met calves had greater body weight (44.1 vs.  $42.1 \pm 0.70$  kg), hip height (81.3 vs.  $79.6 \pm 0.53$  cm) and wither height (77.8 vs.  $75.9 \pm 0.47$  cm). Regardless of colostrum source, the greater body weight, hip height, and wither height in Met calves at birth persisted through 9 weeks of age, resulting in average responses of +3.1 kg body weight, +1.9 cm hip height, and +1.8 cm wither height compared with Controls. Average daily gain during the 9 weeks was  $0.72 \pm 0.02$  kg/d in Met calves compared with  $0.67 \pm 0.02$  kg/d in Control calves. Respiratory scores were normal and did not differ due to maternal Met supply or colostrum source. However, fecal scores tended to be lower in Met calves regardless of colostrum source.

**Table 9.** Weekly growth parameters (1-9 weeks of age), daily starter intake and average daily gain (1-56 d of age) in calves born to Holstein cows offered a control diet (CON) or CON supplemented with ethyl-cellulose rumen-protected Met (Mepron® at 0.09% of diet DM; Evonik Nutrition & Care GmbH, Germany) during the last 28 d of pregnancy.  
<sup>ab</sup>Means differ ( $P \leq 0.05$ ).

Item	Maternal diet		Colostrum type		SEM	P value <sup>1</sup>	
	CON	MET	CON	MET		M	C
Body weight, kg	59.3 <sup>b</sup>	62.4 <sup>a</sup>	61.1	60.5	1.9	0.02	0.66
Hip height, cm	86.9 <sup>b</sup>	88.8 <sup>a</sup>	87.5	88.1	0.68	<0.01	0.34
Hip width, cm	20.3	20.6	20.3	20.6	0.32	0.26	0.17
Wither height, cm	82.7 <sup>b</sup>	84.5 <sup>a</sup>	83.3	83.9	0.67	<0.01	0.31
Body length, cm	126	128	127	126	1.01	0.17	0.68
Daily starter intake, kg	0.79	0.85	0.80	0.84	0.09	0.19	0.39
Average daily gain, kg	0.67 <sup>b</sup>	0.72 <sup>a</sup>	0.68	0.71	0.02	0.03	0.21
Rectal Temperature, °C	38.3	38.7	38.4	38.7	0.26	0.32	0.39
Fecal score <sup>2</sup>	1.83 <sup>a</sup>	1.71 <sup>b</sup>	1.80	1.74	0.09	0.07	0.34
Respiratory score <sup>3</sup>	1.08	1.06	1.07	1.06	0.03	0.61	0.79

<sup>1</sup>Effect of maternal diet (M) and colostrum type (C). There was a time effect ( $P < 0.01$ ) for all these measurements except respiratory score. None of the potential two-way interactions were statistically significant ( $P > 0.10$ ).

<sup>2</sup>Fecal score based on appearance: 1 = Firm well formed; 2 = Soft, pudding like; 3 = Runny, package batter; 4 = Liquid, splatters.

<sup>3</sup>Respiratory score based on appearance: 1 = Normal; 2 = Runny rose; 3 = Heavy breathing; 4 = Cough moist; 5 = Cough dry.

In addition to the differences in calf development and postnatal growth, earlier studies focusing on the effects of enhanced maternal Met supply in late-pregnancy or in the first 70 days post-partum revealed a number of molecular alterations in the embryo or neonatal calf (**Table 10**). Whether those molecular changes involve epigenetic events remains to be determined. However, the fact that they encompass biological processes beyond metabolism (for example, immune function) seems to underscore novel biological roles for Met, and potentially other methyl donors. Further epigenetic analysis, e.g. DNA methylation, of these targets could provide more concrete evidence for a link with met supply. Of particular relevance is the long-term functional ramifications of these molecular changes in terms of health, fertility, and milk production ability.

**Table 10.** Summary of selected studies evaluating the link between enhanced methionine supply and potential programming effects on whole embryo, liver, or innate immune cells.

Objective	Key biological responses	Reference
Evaluate the effect of maternal rumen-protected methionine supplementation during the first 70 days of lactation on the transcriptome of bovine pre-implantation embryos	Increasing methionine supply altered expression of genes related to embryonic development (e.g., VIM, IFI6, BCL2A1, and TBX15) and immune response (e.g., NKG7, TYROBP, SLAMF7, LCP1, and BLA-DQB) in pre-implantation embryos	Peñagaricano et al. 2013. PLoS One. 8(8):e72302.
Determine the effect of maternal rumen-protected methionine during late-pregnancy on blood biomarkers and the liver metabolic transcriptome in neonatal calves	Transcriptome results indicated that calves from methionine-supplemented cows underwent a faster maturation of gluconeogenesis and fatty acid oxidation in the liver, which would be advantageous for adapting to the metabolic demands of extrauterine life	Jacometo et al. 2016. J. Dairy Sci. 99(8):6753-6763.
Determine the effect of maternal rumen-protected methionine during late-pregnancy on blood biomarkers and hepatic 1-carbon metabolism	Transcriptome data indicates that calves from methionine-supplemented cows underwent alterations in Met, choline, and homocysteine metabolism partly to synthesize taurine and glutathione, which would be advantageous for controlling metabolic-related stress	Jacometo et al. 2017. J. Dairy Sci. 100(4):3209-3219.
Determine the effect of maternal rumen-protected methionine during late-pregnancy on immune function and epigenetic markers in blood neutrophils	microRNA and mRNA data from isolated blood neutrophils indicated that maternal methionine could alter innate immune function via changes in abundance of targets associated with cell adhesion and chemotaxis, oxidative stress, Toll-like receptor signaling, and Met metabolism	Jacometo et al. 2018. J. Dairy Sci. 101(9):8146-8158.

## Current Limitations, Outstanding Issues, and Future Research

It is evident that nutrition and the metabolic, or stress state of the cow during late-pregnancy can impact the final stages of development of the calf, which in turn can induce chronic changes in growth, metabolism, and immune function. Clearly, these three factors are interrelated, and we often rely on nutrition to tailor the metabolism of the cow in a way that minimizes stress. Given the impact of micronutrients and functional nutrients such as methyl donors, on key pathways related to cow health (e.g. oxidant, inflammator , and immune status) and the programming of the calf, there is urgent need to define “adequacy thresholds” for those compounds. An example of that is the work our lab has performed with methyl donors, particularly Met supply during the periparturient period. At the onset of those efforts we sought to enhance Met supply to evaluate changes in the antioxidant and inflammatory state of the cow (Osorio et al., 2013a). Our guide was to use the well-established lysine to Met ratio of 3:1 in the MP to formulate experimental diets in terms of the amounts (grams per day) that cows were fed (Osorio et al., 2013a). Although using that relationship yielded novel physiological responses (recall this “ideal” ratio was determined in lactating cows) and confirmed the original hypothesis (Met supply enhances antioxidant synthesis), the “optimal” levels of Met, other essential AA, and micronutrients for cows in the periparturient period is still unknown. It is expected that the actual grams of these nutrients that must be supplied beyond what the basal diet (or microbes) provides will differ according to the characteristic of the basal diet (e.g. forage type). However, the lack of knowledge in terms of functional nutrient adequacy is a major current limitation.

One of the outstanding issues pertaining to the concept of “feeding for dual purpose with dual benefit” is to what extent molecular or epigenetic effects that have been reported in relation to maternal nutrition in dairy cows translate to long-term changes in production phenotypes. It is also unknown the degree to which changes in DNA methylation might be associated with micronutrients or functional nutrients. Clearly, fertility and milk production are two of the most important outcomes for the dairy industry and both are related to health. Application of “high-throughput” or “next-generation” DNA sequencing technologies to map the entire “epigenome” would be helpful in potentially identifying CpG islands in specific genomic regions. Although it remains to be determined, the potential exists to identify loci (or genetic markers) in the fetus (or embryo) that are altered by maternal diet or the supply of specific functional nutrients at an epigenome level.

There also needs to be greater emphasis in the future on assessing the role of nutritional programming of the young calf during the neonatal or pre-weaning period. In the context of nutrition and developmental programming, there is evidence that improving the pre-weaning plane of nutrition of the dairy calf can serve as a major environmental factor influencing the expression of the genetic capacity of the animal for milk yield once it enters lactation (Soberon et al., 2012). Doubling the birth weight by 60 days of age through changes in rate and length of feeding of a higher protein (28% crude protein) milk replacer was significantly correlated with first-lactation milk yield, such that for every 1 kg of pre-weaning average daily gain, heifers produced on average 850 kg more milk during their first lactation. Some of those responses could be related with enhanced mammary development as demonstrated in studies comparing control (0.45 kg/d; 20% crude protein, 20% fat milk replacer) or “enhanced” milk replacers (1.13 kg/d; 28% crude protein; 25% fat) during the pre-weaning phase (Geiger et al., 2016). Clearly, there are unique opportunities for enhancing the productive efficiency of dairy cattle through nutrition in utero or prior to weaning. Additional research should try to establish mechanisms and also explore the unique roles of micronutrients and functional nutrients.

The role for “vertical transfer” of microbes from mother into the fetal hindgut is an unexplored area that merits greater emphasis. The hindgut microbiome in the neonate is crucial for proper regulation of host metabolism, immune response and other key physiological processes via the production of numerous bioactive metabolites, such as volatile fatty acids, essential AA, vitamins and neurotransmitters that can impact signaling pathways and metabolism (Thursby and Juge, 2017). There is growing recognition that these coordinated processes could promote growth and development in dairy calves (Malmuthuge and Guan, 2017). Whether it can be programmed during pregnancy or early life in ruminants remains largely unknown.

## **Conclusions**

This review highlights the need for additional work establishing the adequacy of functional nutrients during pregnancy and to perform epigenome analyses to provide the mechanistic insights required in the field of nutritional programming of dairy cattle production and health. This is best exemplified by the extent of knowledge in epigenetic programming through micronutrients and functional nutrients in non-ruminant species and small ruminants (mainly sheep). Because of the obvious importance of dairy cattle to worldwide agriculture and as a source of nutrition for humans, more emphasis should be placed on studying traits of economic importance where

animals are offered more thoughtfully formulated diets that facilitate the study of specific micro and functional nutrients. In that context, it would also be important to develop robust technologies to “protect” functional nutrients from ruminal metabolism while allowing a high bioavailability at the level of the small intestine.

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# **The Effects of Nutrient Supply during Gestation on Maternal, Fetal, and Postnatal Outcomes in Ruminants: Emphasis on Early Pregnancy**

## **Les effets de l'apport en nutriments durant la gestation sur les résultats maternels, fœtaux et postnataux chez les ruminants**

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### **Abstract**

Managing nutrient supply to match demand is critical for sustainable and efficient livestock production. Concepts of developmental programming, or the idea that stressors during critical windows of development can have both short- and long-term consequences in offspring, began to emerge 3 decades ago based on human epidemiological studies. Research with animal models, including livestock, has since demonstrated that developmental programming is probably universal, and that consequences on offspring growth, development, and health are likely much larger than previously realized. Compromised offspring may have altered metabolic and body composition outcomes at various points postnatally, which could influence nutrient requirements. Suboptimal maternal nutrition can alter body composition in offspring at various stages postnatally. Most published research has investigated the influences of suboptimal maternal nutrition during the last two-thirds of gestation. Emerging data indicate that maternal nutrition during early pregnancy may be much more important to the developing conceptus than previously thought. Additional research in the area of maternal nutrition and offspring outcomes will provide new knowledge that could lead to altered management practices and increased efficiency of ruminant production.

### **Résumé**

Gérer l'apport en nutriments pour répondre à la demande est essentiel à la production de bétail durable et rentable. Les notions de programmation de la croissance, où l'idée que les stressors durant les phases critiques de croissance peuvent avoir à la fois des conséquences à court et à long terme sur la progéniture, ont commencé à émerger il y a une trentaine d'années dans les études sur l'épidémiologie humaine. La recherche avec des modèles animaux, incluant le bétail, a depuis démontré que la programmation de la croissance est probablement universelle et que les conséquences sur la croissance, le développement et la santé de la progéniture sont vraisemblablement plus importantes qu'on ne le pensait. La progéniture compromise peut montrer des résultats métaboliques et de composition corporelle altérés à divers moments après la naissance, ce qui pourrait influencer les exigences de nutriments. La nutrition maternelle sous-optimale peut altérer la composition corporelle de la progéniture à divers stades suivant la naissance. La plupart des recherches publiées ont étudié les influences de la nutrition maternelle sous-optimale pendant les deux derniers tiers de la gestation. Les nouvelles données indiquent que la nutrition maternelle en début de grossesse peut être plus importante pour le produit de conception en croissance qu'on

ne le croyait auparavant. D'autres recherches sur la nutrition animale et sur les résultats de la progéniture apporteront de nouvelles connaissances qui pourraient mener à des pratiques de gestion repensées et à une production de bovins de boucherie plus rentable.

## **Introduction**

Animal agriculture faces immense challenges in the near future. The FAO (2017) maintains that livestock contribute 15% of total food energy, 25% of dietary protein, and provide essential micronutrients not easily obtained from plant food products. In the U.S. alone, production of animals for food and fiber is a multi-billion dollar industry. Currently, livestock contributes 40% of the global value of agricultural production and supports the livelihoods and food security of almost 1 billion people (FAO, 2017). However, the world needs to significantly increase its output of meats by 2050 and beyond to meet the projected requirements of the rapidly growing population (Elliot, 2013; FAO, 2017). Consequently, efficient and sustainable approaches to livestock production are essential.

Feed costs are the largest economic burden for beef cattle producers. For beef cattle, more feed resources are dedicated to the parent population (cow herd) than to market bound offspring (Webster, 1989). In mature beef cows, approximately 70% of dietary energy is consumed by maintenance functions (Jenkins and Ferrell, 1983; Ferrell and Jenkins, 1985; Ferrell, 1988; NASEM, 2016). Maintenance energy is consumed extensively by visceral tissues and muscle (Ferrell, 1988; Baldwin and Donovan, 1998; Caton et al., 2000). Small changes in efficiency of energy use for maintenance equate to considerable energetic savings and represent opportunities for enhanced production efficiencies (NASEM, 2016).

Metabolically and otherwise compromised animals are major detriments to efficient, sustainable livestock production systems (Reynolds and Caton, 2012). Muscle and viscera (liver and gut) are key tissues for feed efficiency because they use large amounts of nutrient resources. Muscle and liver tissues are likely programmed during development (Caton et al., 2018; Crouse et al., 2019 a,b). Therefore, improvements in fetal development leading to better pregnancy outcomes and offspring performance postnatally would have a significant positive impact on the major societal issues of feeding the growing world population and enhance the competitiveness, sustainability, and profitability of animal agriculture.

## **Maternal Nutrition and Developmental Programming**

Developmentally compromised offspring have an increased risk of poor production and health complications throughout life (Wu et al., 2006; Caton and Hess, 2010; Reynolds and Caton, 2012; Meyer and Caton, 2016; Reynolds et al., 2017). The concept of developmental programming was originally based on epidemiological studies in humans, but strong evidence has accumulated in livestock. Compromised maternal nutrient supply can cause developmental programming events and consequently alter offspring outcomes (Wallace et al., 1999; Wu et al., 2006; Caton and Hess, 2010; Funston et al., 2012; Reynolds and Caton, 2012; Robinson et al., 2013; Reynolds and Vonnahme, 2017). Fetal growth and development are affected by maternal nutrient intake, even during very early stages of development when nutrient requirements for conceptus growth are reported to be negligible (Robinson et al., 1999; NRC, 1996, 2007; Reynolds et al., 2014;

NASEM, 2016). Maternal nutrient restriction that results in decreased fetal nutrient supply during critical developmental windows (Caton and Hess, 2010; Reynolds and Caton, 2012) may arise from various events, including altered maternal nutrient supply, placental dysfunction, compromised maternal metabolism, physiological or environmental extremes, or combinations of a multitude of situations.

Offspring that are growth restricted during development are at risk of postnatal complications, which may result in poor growth and development, as well as poor productivity and reduced longevity later in life (Wu et al., 2006; Caton and Hess, 2010; Funston et al., 2012; Reynolds and Caton, 2012). Compromised maternal nutrition and restricted fetal growth are associated with reduced growth efficiency and altered body composition (Greenwood et al., 1998, 2000; Wu et al., 2006; Caton et al., 2007; Larson et al., 2009; Robinson et al., 2013). Birth weights in cattle are related to postnatal growth performance (Robinson et al., 2013; NASEM, 2016), and maternal nutrient restriction can alter composition of offspring growth in the absence of birth weight differences (Reynolds and Caton, 2012). In addition, energy requirements of offspring may be altered by developmental programming events (Caton et al., 2018). Individuals with altered postnatal metabolism or growth can result in management challenges for livestock producers because nutritional management decisions are often based on the averages of groups of animals. Management decisions that reduce the negative aspects of developmental programming may improve efficiency of ruminant livestock production, which will address the challenge of doubling livestock production to feed the projected world population of 9.6 billion by the year 2050 (Elliot, 2013; Reynolds et al., 2015; United Nations, 2015).

## **Importance of Early Pregnancy**

Nutrient requirements for pregnancy increase as gestation advances (NASEM, 2016); however, starting very early in pregnancy, nutrient supply to the developing conceptus is critical for survival and growth. Most large-animal models of developmental programming focus on insults (compromised nutrition and/or other issues) during mid- to late gestation and the resulting effects on offspring. During early gestation, the conceptus grows from one cell to a fully formed embryo with recognizable organ systems in 35 to 50 days. In addition, during this time the placenta has to grow and develop to functionality. Tissue doubling rate during this stage of gestation is very rapid. Consequently, nutrient supply needs to support rapid growth and development to ensure embryonic survival and establishment of pregnancy. During the early phase of fetal development, differentiation and vascularization of utero-placental tissues, as well as fetal organogenesis occur, all of which are critical events for normal fetal development (Funston et al., 2010). Additionally, dams that undergo stress (nutritional, environmental, etc.) during early, but not late gestation, are likely to produce normal birth weight offspring that still suffer from poor growth and metabolic complications because of the stress early in pregnancy (Ford et al., 2007; Vonnahme et al., 2007; Reynolds and Caton, 2012).

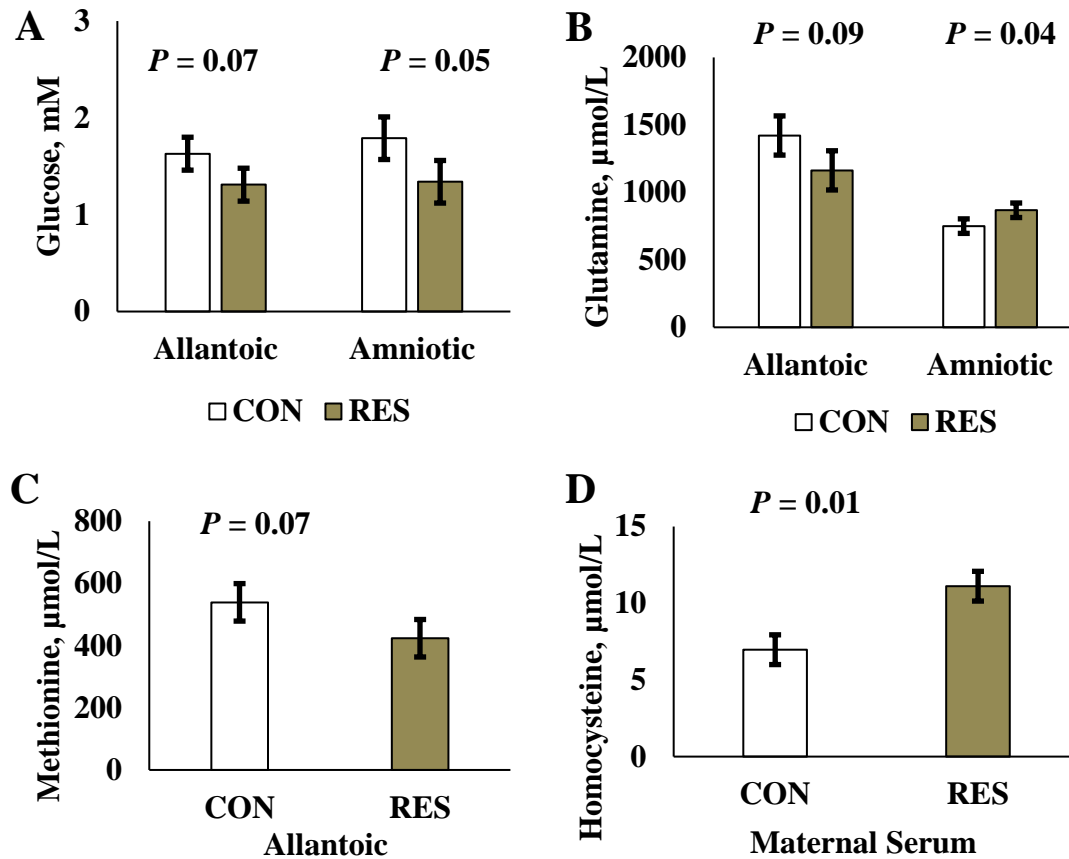
Recently, we developed an ovariectomy technique (McLean et al., 2016) to investigate developmental programming responses to moderate nutrient restriction during the first 50 d of pregnancy in beef cattle. In these studies, post-pubertal heifers were fed to gain 0.45 kg/d (control) or 0 kg/d (moderate restriction) for the first 50 d post-breeding. At various times during early pregnancy, ovariectomies were conducted and tissues collected. Actual rates of gain were

0.5 vs. -0.08 kg/heifer daily for control and moderate restriction, respectively. Results from these studies demonstrated nutrient and metabolite changes in fetal fluids (Fig. 1). For example, at d 50 of gestation glucose, methionine, and glutamine were decreased in allantoic fluids in moderately restricted heifers. Amniotic fluid glucose was also decreased, whereas amniotic glutamine was increased in moderately restricted heifers. Maternal serum homocysteine also was increased in the moderately restricted heifers, suggesting compromised one-carbon metabolism (Crouse et al., 2019a).

Data from these studies also investigated gene expression in fetal muscle from the hind limb and fetal liver at d 50 of gestation. In fetal liver and muscle we found a total of 548 and 317 genes, respectively, were differentially expressed as a result of moderate maternal nutrient restriction. Of these, 201 and 144 genes, respectively, were false discovery rate-protected (Crouse et al., 2019b). Pathway analysis was performed on the differentially expressed genes to determine the functional categories of pathways or ontologies associated with factors known to affect production efficiencies (Table 1; Crouse et al., 2019b). In fetal liver, five functional categories of interest were affected by moderate nutrient restriction during the first 50 days of gestation were (Crouse et al., 2019b): metabolic pathways (n = 43 genes); protein kinases (n = 47 genes); nucleosome core proteins (n = 22 genes); mRNA splicing (n = 7 genes); and complement/coagulation cascades (n = 6 genes). In fetal muscle, three functional categories of interest were affected by moderate nutrient restriction: skeletal muscle (n = 74 genes); embryogenesis (n = 14 genes); and signaling cascades (n = 18 genes). These changes in gene expression could be indicative of differential epigenetic programming and potentially postnatal efficiency and growth. It is particularly interesting that 78% of differentially expressed genes were upregulated in offspring gestating in dams receiving moderate nutrient restriction.

## Summary and Conclusions

In summary, animal agriculture faces major challenges in regards to feeding the growing world population. Increasing production efficiencies in ways that are more sustainable and that foster healthy and robust offspring will substantially contribute to meeting world food demands and at the same time foster sustainability of agricultural systems. Appropriate maternal nutrient supply is critical for health, well-being, and productivity of both the dam and offspring. Inappropriate maternal nutrition can result in negative developmental programming events that may compromise offspring in both the short- and long-term. Most published research investigating developmental programming events has focused on maternal nutrition during the last two-thirds of pregnancy. Emerging evidence are taken to imply that moderate changes in maternal nutrition during early pregnancy will elicit developmental programming events. Long-term consequences of these early programming events remain to be determined.



**Figure 1.** Comparison of (A) glucose concentrations in allantoic and amniotic fluid, (B) glutamine concentrations in allantoic and amniotic fluid, (C) methionine concentration in allantoic fluid, and (D) homocysteine concentration in maternal serum of heifers receiving control (CON) or restricted (RES) dietary treatment from the day of mating (d 0) until d 50 of gestation. Treatments provided for 0.5 kg vs. -0.08 kg of gain/heifer daily between d 0 and 50 of gestation for CON vs. RES heifers, respectively (Adapted from Caton et al., 2018 and Crouse et al., 2019a).

**Table 1.** Functional categories and predicted roles for differentially expressed genes that impact production efficiencies ( $P < 0.01$ ) in fetal liver and muscle from hind limb (adapted from Crouse et al., 2019b).

Tissue	Category	Functional annotation <sup>1</sup>	Total genes <sup>2</sup>	RES <sup>3</sup>	CON <sup>4</sup>	<i>P</i> -value <sup>5</sup>
Liver	Metabolic pathways	Amino acid	10	5	5	0.017
		Purine and pyrimidine	7	7	0	
		Carbohydrate	10	5	5	
		Reducing equivalent	5	5	0	
		Steroid and lipid biosynthesis	9	8	1	
		Cytochrome and heme	2	2	0	
	Protein kinase	Ser/Thr protein kinase	22	21	1	0.020
		ATP-binding	19	15	4	
		Nucleotide-binding	6	4	2	
	Nucleosome core	Histones	9	9	0	0.005
		Histone modifiers	13	12	1	
	mRNA splicing	Spliceosome	7	6	1	0.041
Muscle	Skeletal muscle	Contraction	9	9	0	0.001
		Intermediate filament	11	7	4	
		Microtubule	10	2	8	
		Actin	4	3	1	
		Myosin	4	4	0	
		Troponin	6	6	0	
		Calcium-binding	25	14	11	
		ATP-binding	5	0	5	
	Embryogenesis	Myogenesis	2	2	0	0.001
		Homeobox	12	10	2	
	Signaling cascades	Wnt	6	4	2	0.003
		MAPK	12	3	9	

<sup>1</sup>Proposed function of differentially expressed genes that fall under a specific category.

<sup>2</sup>Total number of differentially expressed genes associated with a specific function.

<sup>3</sup>Number of differentially expressed genes that are upregulated in RES fetuses.

<sup>4</sup>Number of differentially expressed genes that are upregulated in CON fetuses.

<sup>5</sup>Probability value associated with a specific category.

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## Interaction of Nutrition and Epigenetic Effects in Dairy Cattle

### Interaction entre la nutrition et les effets épigénétiques chez les bovins laitiers

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#### Abstract

The dairy industry has achieved astonishing improvements in the last decades through bold progress in genetics, nutrition, and management. In the last years a new possibility for further improvement is emerging through the understanding of the mechanisms involved in the control of the expression of the genetic potential of a given individual. In adult dairy cows, close to 70% of the gestation coincides with lactation, and during this time the placenta must compete for nutrients with the mammary gland. It is now known, that the nutritional status of the pregnant cow exerts important influence in the regulations of the expression of the genetic potential of the offspring. These changes in expression may result in both positive and negative outcomes. The cluster of mechanisms that modulate gene expression are referred to as epigenetics, and they include DNA methylation, histone modifications (i.e., acetylation, methylation, phosphorylation, ubiquitination, and sumoylation), and short noncoding RNA fragments called microRNA (miRNA). Epigenetic influences have been described as a consequence of the nutrient supply and hormonal signals at which the offspring is exposed at specific stages during development, both before and after birth. For example, there are differences in the epigenome of cows born to heifers (non-lactating while pregnant) and those born to cows (lactating while pregnant), and this epigenome is sensitive to the availability of methyl donor compounds of the dam during pregnancy. Another example is the lower milk production at adulthood of calves born to dams that were heat-stressed during the last months of pregnancy compared to those born to dams kept under thermo-neutral conditions. Furthermore, there are indications that plane of nutrition (and rate of growth) of calves during the first stages of life are associated with milk yield at adulthood. In rats, it has been shown that maternal stress in late gestation alters expression of a number of miRNA in the brains of the offspring, and these changes are kept throughout several generations. Epigenetic marks can take place in somatic cells and in germinal cells, and marks occurring in germ cells could potentially be transferred onto next generations. In this regard, an untapped opportunity for further improvement of dairy performance and health, is the potential role of epigenetic inheritance via the paternal line, as there is evidence in rats that the physiological environment of the sire alters a number of miRNA in the offspring targeting expression of genes involved in chromatin remodeling. It is foreseeable that the dairy industry will embrace this new opportunity to improve animal performance and health once the underlying mechanisms are fully uncovered.

## Résumé

L'industrie laitière a réalisé des améliorations stupéfiantes depuis quelques décennies, grâce à des progrès audacieux dans les domaines de la génétique, de la nutrition et de la gestion. Ces dernières années, une nouvelle possibilité d'amélioration encore plus poussée émerge avec la compréhension des mécanismes contrôlant l'expression du potentiel génétique d'un individu donné. Chez les vaches laitières adultes, près de 70 % de la gestation coïncide avec la lactation et, pendant ce temps, le placenta doit concurrencer avec la glande mammaire pour obtenir ses nutriments. On ne sait pas dans quelle mesure le statut nutritionnel de la vache en gestation influence les régulations de l'expression du potentiel génétique de la progéniture. Ces changements dans l'expression peuvent produire tant des résultats positifs que négatifs. La grappe de mécanismes modulant l'expression génétique s'appelle l'épigénétique, alors que les mécanismes incluent la méthylation de l'ADN, les modifications de l'histone (c.-à-d. acétylation, méthylation, phosphorylation, ubiquitination et sumoylation) ainsi que les fragments courts d'ARN non codant, appelés micro-ARN (miARN). On a décrit les influences de l'épigénétique comme une conséquence de l'apport en nutriments et des signaux hormonaux auxquels la progéniture est exposée à des stades donnés de son développement, avant et après sa naissance. Par exemple, il y a des différences dans l'épigénome des vaches nées de génisses (non lactantes en gestation) et celles nées de vaches (lactantes en gestation) et cet épigénome est sensible à la disponibilité des composés du donneur méthyle du géniteur mâle durant la grossesse. Un autre exemple est la plus faible production de lait des veaux adultes nés de géniteurs mâles soumis au stress thermique durant les derniers mois de la grossesse, comparativement à ceux nés de géniteurs mâles gardés sous conditions thermiques neutres. Qui plus est, il y a des indications à l'effet que le niveau de nutrition (et le taux de croissance) des veaux durant les premiers stades de la vie sont associés au rendement du lait à l'âge adulte. Chez les rats, on a observé que le stress maternel en fin de gestation altère l'expression d'un certain nombre de miARN dans les cerveaux de la progéniture, alors que ces changements subsistent pendant plusieurs générations. Des marques épigénétiques peuvent s'inscrire dans les cellules somatiques et les cellules germinales; les marques des cellules germinales pourraient potentiellement être transférées aux générations suivantes. À cet égard, une opportunité inexplorée d'améliorer encore le rendement laitier et la santé, c'est le rôle potentiel du patrimoine physiologique du reproducteur à travers la ligne paternelle, alors qu'on a observé chez les rats que l'environnement physiologique du reproducteur altère plusieurs miARN dans la progéniture, ciblant l'expression des gènes qui participent au remodelage de la chromatine. L'industrie laitière profitera fort probablement de cette nouvelle opportunité d'améliorer le rendement et la santé des animaux une fois que l'on connaîtra tous les mécanismes sous-jacents.

## Introduction

Jean-Baptiste de Lamarck (1744-1829) was the first to propose, in his *Philosophie Zoologique* (1809) a clearly evolutionary theory. He questioned the fixity of species when he was working as curator of invertebrates at the *Muséum d'Histoire Naturelle* in Paris. He proposed that primitive organisms were generated spontaneously and then changed progressively along the 'Chain of Being'. A few years later, Darwin rejected Lamarck's belief (Darwin, 1868), and the Lamarckian theory of inheritance was explicitly rejected by the German biologist August Weissmann (1834-1914). He made the distinction between soma and germen and postulated that from the fertilized

egg there are two independent processes of cell division, one leading to the body or soma and the other, the germ line, leading to the gametes that constitute the starting point of the next generation. The soma inevitably dies when the organism dies, but reproduction is concentrated in the germ line potentially immortal. Hence, selection between cell lines is possible. There are several examples, however, where some evidence that the effects of environment on the phenotype are inherited throughout some generations. For example, Waddington (1942) exposed *Drosophila* embryos to ether, which induces the phenotype bithorax in adult flies (four wings instead of two). After many generations of ether exposure and artificial selection for the bithorax phenotype, the selected flies had the aberrant phenotype even when they were not exposed to ether. This phenomenon is known as genetic assimilation (Waddington, 1953) where an initially-acquired trait can become genetically propagated. The phenomenon of genetic assimilation is sometimes questioned as an example of Lamarckian inheritance, and it is rather described as a genetic stabilization of a phenotype that was previously induced by an environmental stimulus and was considered to be an epigenetic effect. The word 'epigenetic' was introduced by Waddington (1942), derived from the Aristotelian word *epigenesis*. Initially, epigenetic was defined as the causal interactions between genes and their products which bring the phenotype into being. At the time of Waddington's experiments, genetics was an infant field, and little knowledge of the genome and its biology existed. Nowadays, it is such a broad field that there is no consensus on the current definition of the term (Deans and Maggert, 2015; Holliday, 2006). Epigenetic includes any modification on the expression of genes that is due to factors other than a mutation in the DNA. This involves DNA methylation, post-translational modification of histones, regulation of gene expression by non-coding RNAs, and genome instabilities or any other force that could modify a phenotype.

One of the most intriguing and promising aspects of the potential transgenerational role of epigenetics resides in the cellular energetic status. Caloric energy is an important factor in the cellular environment that can influence cellular gene expression, DNA replication, growth, proliferation, differentiation, and even programmed death. The high mutation rate of the mitochondrial DNA (mtDNA) and its direct effect on bioenergetics make the mtDNA an excellent genetic system for the adaptation of species subpopulations to regional differences in their energetic environment (Wallace and Fan, 2010). In fact, it has been proposed that epigenetic mechanisms that regulate the expression of nuclear genome influence mitochondria by modulating the expression of nuclear-encoded mitochondrial genes, and that a cell-specific mitochondrial DNA content (copy number) and mitochondrial activity can determine the methylation pattern of nuclear genes (Manev and Dzitoyeva, 2013). While most epigenetic marks are deleted during the meiosis, mutations in the mtDNA may induce long-lasting mutations in the genomic DNA. For instance, some cancer studies have shown that mitochondrial dysfunction is associated with epigenetic alteration within the nuclear genome (Xie et al., 2007; Smiraglia et al., 2008). If these mutations were to occur in the gametes, then the influence of the environment on the adaptation of species through epigenetics mechanisms would be plausible. For instance, the ovary actively selects against those oocytes that contain lethal mutations in the mtDNA, but those that undergo unnoticed will not manifest after development results in complex tissues and organs.

Nutritional models used to assess nutrient requirements of dairy cattle have focused on maximizing production performance and attempting to ameliorate the array of metabolic afflictions that cows typically undergo after calving, but, with the exception of copper, omit any potential nutrient

requirement for pregnancy until 190 days of gestation. The main reason for this is that fetal weight increases exponentially during gestation and it is only during the last stages of pregnancy that fetal weight is considered to be sufficiently large to start accounting for nutrient needs. However, nutrient supply and hormonal signals at specific windows during development (both pre- and early post-natal) may result in permanent changes in body composition and metabolic function of the offspring of livestock (Wu et al., 2006), through processes generically referred to as ‘fetal programming’ and ‘metabolic imprinting’. These long-term modifications of the metabolic function of the offspring are thought to occur through epigenetic changes (Anderson et al., 2006; Wu et al., 2006), which primarily involve modifications in the chromatin structure through the acetylation of histones or methylation of DNA, resulting in modulation of gene expression independent of gene sequence.

Lastly, it must be beard in mind that two types of epigenetic inheritance are usually referred to: 1) epigenetic marks, which can be inherited in the soma line as these marks are conserved during mitosis (Jablonka and Raz, 2009), and 2) transgenerational epigenetic inheritance via the germ line, which controls patterns of gene expression that are passed from one generation to the next by molecules in the gametes (Daxinger and Whitelaw, 2012).

### **Epigenetics before birth (fetal programming)**

Potential causes and consequences of fetal programming have been reviewed in both beef (Funston et al., 2010) and dairy (Bach, 2012) cattle. González-Recio et al. (2012) reported that, when accounting for the genetic merit of the dams and sires, cows born to mothers that were lactating while pregnant (adult cows) produced less milk, lived shorter and were metabolically less efficient than cows born to dams that were not lactating while pregnant (heifers). The mechanisms by which these phenotypic observations are brought about are unknown, but it is likely that the metabolic status of the lactating dam may exert some epigenomic effect at the level of both the oocyte and embryo. In fact, Bach and Aris (2013) explored potential differences in the degree of methylation of the entire genome of calves born to primiparous (no coexistence of lactation and pregnancy) and calves born to multiparous (coexistence of lactation and pregnancy) cows and identified 70 regions of the genome of the offspring that had a different methylation status depending on the parity group of the dam. Of these 70 regions, one corresponded to the gene coding for zona pellucida protein 1, which participates in the fertilization of the oocyte (Rankin et al., 1999) and was methylated in daughters of multiparous cows and unmethylated in daughters of primiparous cows. Bach and Aris (2013) speculated that this difference may result in worse reproductive performance in the offspring from multiparous than from primiparous cows. Later, (Bach, 2019) used a dataset containing the reproductive records of 56,701 heifers that were reared and fed under identical conditions in a contract heifer operation (Rancho Las Nieves, Mallén, Spain) to evaluate whether this hypothesis could be plausible and reported that the mean ( $\pm$ s.e.) conception rate at first insemination in heifers born to heifers ( $69.6 \pm 0.1\%$ ) was greater ( $P < 0.01$ ) than that of heifers born to lactating cows ( $67.3 \pm 0.1\%$ ).

In some instances, lactating cows become pregnant while in negative energy balance, or at least with relatively high blood NEFA concentrations. Elevated blood NEFA concentrations in the oocyte’s microenvironment can affect gene expression. For example, cumulus cells exposed to high NEFA concentrations exhibited downregulated expression of DNA methyltransferase 3 alpha (DNMT3A;

Van Hoeck et al., 2013), which is involved in de novo methylation of cytosine residues at cytosine–phosphorous–guanine (CpG) sites in oocytes and early preimplantation embryos (Uysal et al., 2015). Furthermore, Desmet et al. (2016) reported differences in gene expression and methylation in oocytes exposed to high compared with physiological concentrations of NEFA and that these changes were more evident during the in vitro culture stage than during maturation.

In addition, the protein nutritional status of the dam may have long-term effects on the reproductive ability of the offspring. For example, in primiparous beef cows, a protein deficiency during the last 100 days of pregnancy delayed age at puberty of the progeny (Corah et al., 1975). Similarly, heifers born to dams supplemented with protein during the last one-third of pregnancy had increased pregnancy rates compared with heifers born to unsupplemented dams (Martin et al., 2007). Similarly, the nutritional status of the dam in terms of methyl donor availability may also have some epigenetic consequences in the offspring. Epigenetics depend, in part, on methyl donor availability, and thus, a shortage of methyl donors in the dam could result in some epigenetic changes in the offspring. Targeted dietary supplementation with folate, choline or betaine appears to consistently increase DNA methylation because these nutrients are methyl donors (Anderson et al., 2012). In addition, B vitamins, such as vitamins B<sub>2</sub>, B<sub>6</sub> and B<sub>12</sub>, appear to increase DNA methylation because of their role as cofactors in the methylation process (Craig 2004). Cordero et al. (2013) described epigenetic changes in the offspring of rats fed high-fat and -sucrose diets, but these epigenetic marks were reversed by supplementation with a methyl donor (folic acid and vitamin B<sub>12</sub>). In dairy cattle, Gagnon et al. (2015) reported that intramuscular injections of folic acid and vitamin B<sub>12</sub> during the 24 days before and 56 days after calving had minor consequences on milk yield, but they reported several changes in the expression of several genes in granulosa cells, and provided some support to the hypothesis that lactating cows may not be able to meet their methyl donor need through conventional rations. In fact, Jacometo et al. (2016) reported that supplementing lactating dams with methionine (a methyl donor) resulted in calves that underwent a faster maturation of gluconeogenesis and fatty acid oxidation pathways in the liver, which would be advantageous for adapting to the metabolic demands of extrauterine life, and Bach et al. (2017) evaluated the potential epigenetic effects of supplementing vitamin B<sub>12</sub> and folic acid during pregnancy and reported distinct methylation patterns in the offspring depending on the parity of the dam and supplementation of methyl donors.

### **Epigenetics before conception (paternal effects)**

A potentially important and largely overlooked aspect of fetal programming is the hypothetical effect of paternal epigenetic marks. Although it has been clearly established that the intrauterine environment plays a central role in establishing critical metabolic functions in the fetus, the effect of paternal nutritional or metabolic status during spermatogenesis should not be disregarded (DelCurto et al., 2013). As it occurs with females, changes in DNA methylation, histone modification and non-coding RNAs are all plausible mechanisms for a non-genetic transfer of paternal environmental information from the maturing germ cell to the zygote (Soubry et al., 2014). Studies involving mice have reported paternal obesity reduces rates of blastocyst development and pregnancy success (Mitchell et al., 2011; Binder et al., 2012). Similarly, offspring from fathers fed low-protein diets has been reported to increase hepatic expression of several genes involved in lipid and cholesterol biosynthesis (Carone et al., 2010). Recently, a transgenerational paternal inheritance of

diabetes has been described by comparing the epigenomic patterns of the spermatozoa with those of the pancreatic islets of the offspring (Wei et al., 2014), and Vassoler et al., (2013) reported that changes in epigenetic marks in the paternal germline were transmitted to the male but not female offspring. There is scarce information about the role of paternal epigenetic marks in mammals, and no information in cattle; however, if found relevant, paternal epigenetic marks in dairy cattle could have a large effect on dairy production due to the widespread use of artificial insemination. Furthermore, if the epigenetic variance, or that due to imprinted genes, was sufficiently large, selection on male and female lines could be done separately (Goddard and Whitelaw, 2014). However, a low-cost procedure for epigenome screening would be necessary to implement this type of strategy (González-Recio et al., 2015). Nevertheless, with the progressive implementation of genomics in dairy cattle selection schemes, the incorporation of epigenetic information is likely to significantly improve phenotypic outcomes.

### **Epigenetics after birth conception (metabolic imprinting)**

Epigenetic changes in the cells decrease with age. However, plane of nutrition early in life exerts some long-term effects on the expression of the genetic potential of the animal. For example, the pioneering work of McCance (1962) illustrated that limit-feeding rats during the first 21 days of life resulted in a lifetime programming of growth pattern that was lesser than that of rats fed properly. More interestingly, when the dietary restriction was applied for 21 days but at a more advanced age, the intervention had no lasting effect because the underfed rats showed compensatory growth gains when re-fed at normal levels. However, dairy cattle are much more precocial than rats, an altricial species, and thus, it could be expected that changes in post-natal growth could have a lesser impact than in rats. Nevertheless, some studies have confirmed that a nutritional restriction to weaning age limits compensatory growth in cattle (Café et al., 2006; Greenwood et al., 2006), and others have described that increased average daily gain during the first 2 months of life results in significant y greater body weight at 24 months of age (Robelin and Chilliard, 1989; Moallem et al., 2010), and others (Bach and Ahedo, 2008; Bach, 2012; Soberon et al., 2012) have reported increases in milk production associated with improved daily gains during the first 2 months of life. Also, the type of calving seems to exert an effect on calves. Barrier et al. (2012) reported a greater mortality risk to weaning and to first service in the live-born heifers that experienced moderate difficulty at birth compared with heifers born to unproblematic calvings

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## Developmental Programming in the Beef Industry

### Programmation de la croissance dans l'industrie du bœuf

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#### Abstract

Beef producers have often recognized that one year's calf crop may perform better or worse than the usual herd average. Many of these effects are now thought to be caused by developmental programming interactions. Developmental programming uses stressors, often nutritionally induced, during key windows of the growth and development of the offspring. This results in inherent changes to the genetic potential of the offspring, which has the potential to impact economically relevant traits. This represents an opportunity to create multi-generational nutrition programs to pre-program cattle for improved health, performance, and efficiency. The "thrifty phenotype hypothesis" suggests that maternal nutrient restriction at key junctures may improve overall feed efficiency. Although some work suggests that severe nutrient restriction of 40-60% generates this effect, genetic changes supporting a thrifty phenotype can be observed with a mild restriction of 10% total NE requirements. In addition, previous research in beef cattle has demonstrated nutritional stressors during mid-to late gestation may influence reproductive traits in heifers, improved meat quality, and performance traits in the feedlot. Current work suggests even neonatal developmental programming may occur, as protein supplementation during late gestation resulted in colostrum with different proteomic and lipidomic profiles. Although the science of developmental programming is still relatively new, this nutritional management tool has the potential to revolutionize the way we feed cattle and further support a sustainable beef industry.

#### Résumé

Les éleveurs de bovins de boucherie ont souvent observé que les veaux d'une année donnée peuvent avoir un rendement meilleur ou pire que le troupeau moyen habituel. On pense maintenant que plusieurs de ces effets sont causés par les interactions de la programmation de la croissance. La programmation de la croissance utilise les stressors, souvent induits par la nutrition, durant les phases clés de croissance et de développement de la progéniture. Cela produit des changements inhérents au potentiel génétique de la progéniture, qui peuvent avoir des effets sur des traits liés à l'économie. C'est aussi une opportunité de créer des programmes de nutrition multigénérationnels afin de préprogrammer le bétail et d'améliorer sa santé, son rendement et sa rentabilité. L'« hypothèse du phénotype vigoureux » donne à penser que la restriction des nutriments à la mère à des points charnières peut améliorer l'efficacité globale de ses aliments. Bien que certains travaux portent à croire qu'une restriction sévère des nutriments de 40 à 60 % produise cet effet, des changements génétiques supportant un phénotype vigoureux peuvent être observés, avec une légère restriction

de 10 % des exigences totales de NE. De plus, les recherches antérieures sur le bœuf de boucherie ont démontré que les stressors nutritionnels, du milieu vers la fin de la gestation, peuvent améliorer les traits reproducteurs des génisses, la qualité de la viande et les caractéristiques de rendement du parc d'engraissement. Les travaux actuels donnent à penser que même la programmation de la croissance néonatale peut survenir, alors que la supplémentation protéique en fin de gestation a produit un colostrum montrant différents profils protéomiques et lipidomiques. Bien que la science de la programmation de la croissance soit relativement jeune, cet outil de gestion nutritionnelle a le potentiel de révolutionner la façon dont nous alimentons notre bétail et de mieux soutenir la viabilité de l'industrie du bœuf.

## **Introduction**

In recent years the concepts of developmental (or fetal) programming and epigenetics have become hot topics in research for human medicine, but also have major implications for livestock production. Historically, beef producers have often noted that in certain years calves may perform better or worse than average. Although these are largely anecdotal accounts, these observations are rooted in the rapidly emerging field of developmental programming research. The majority of this work was first confirmed from ground breaking epidemiological data surrounding health outcomes of people and their families, who were born immediately following the “Dutch famine” of World War II. (Rosebloom et al., 2001). In general the concept of developmental programming suggests that early-life stressors (nutritional, environmental, toxicological, etc.) can alter later-in-life phenotype and physiological outcomes, and may even have lasting intergenerational effects (epigenetic effects). The timepoint in which these stressors occur during placental and fetal development influences which biological systems may be most impacted by this stressor and resultant phenotypic modifications. With increasing scientific understanding it is possible that livestock can be managed deliberately to capitalize on these differences for improved production and sustainability.

In this publication we will highlight some advances in developmental programming research in beef cattle which have potential to improve economically relevant traits for beef cattle production. In addition, the concept of post-partum and lactocrine programming will be introduced and recent results from our work in this area will be highlighted. Finally, opportunities and challenges in application of these technologies for the beef industry in Canada will be discussed. Developmental programming continues to be an exciting frontier in scientific discovery and are likely to have tremendous impacts on traditional views of livestock nutritional management.

## **Developmental Programming in Beef Production**

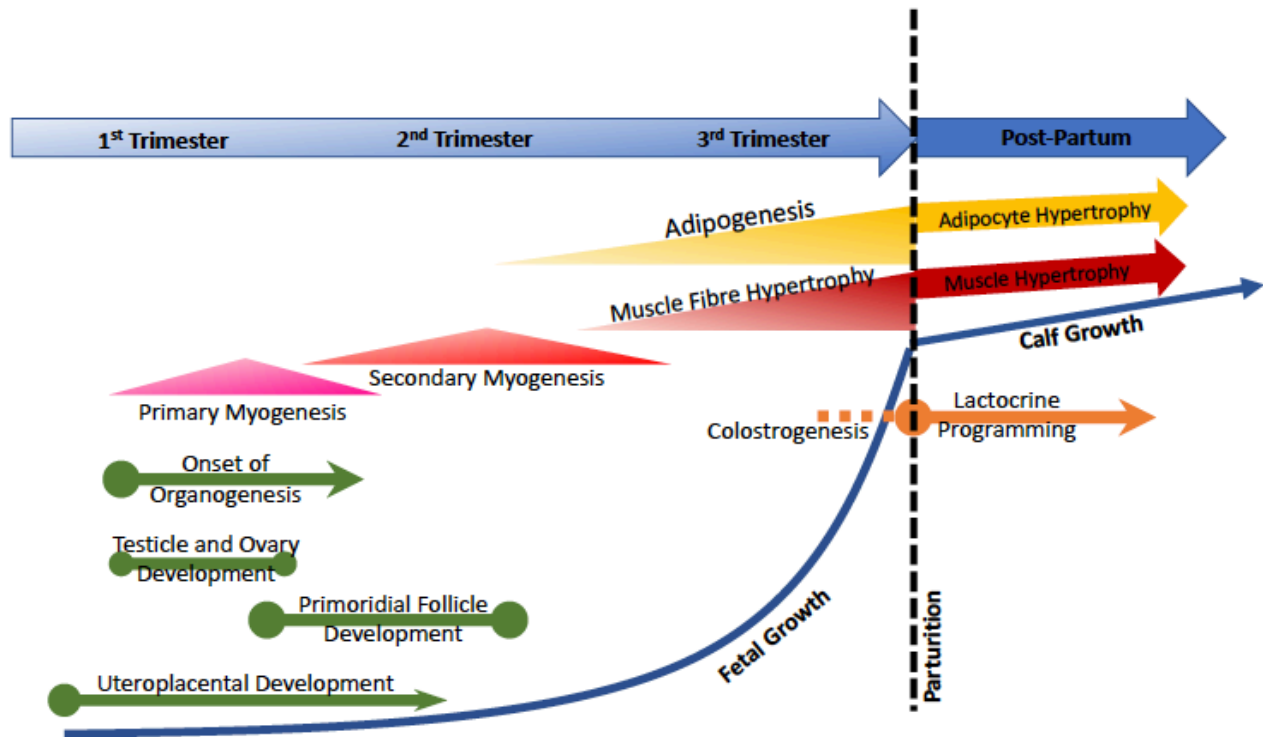
Beef production differs from many of our other domesticated livestock species in that for the majority of the year the breeding herd is managed in extensive environments. Therefore studying developmental programming events in the beef herd has two main directions, a proactive approach and a reactive approach. A proactive approach in beef production is similar to developmental programming strategies investigated in many livestock species, where nutritional management strategies are developed to target key developmental programming outcomes. For example, using protein supplementation in mid-to-late gestation in effort to increase muscle development (Du et al., 2010). These approaches may use nutrient supplementation (Protein, AA, anti-oxidants,

PUFAs, and others) or global nutrient restriction or oversupply to induce a nutritional stress during key windows *in utero*. Equally as important for the beef industry the reactive approach, where understanding the impacts of environmental stressors (and associated induced nutritional stressors) on developmental programming and the implications for management of the resulting offspring. A clear application of this approach is in understanding the impacts of drought on the breeding herd and helping producers make management decisions surrounding management of resultant offspring.

There are many excellent published reviews on the potential of fetal/developmental programming as a transformative technology in the beef industry, including Funston et al., (2010); Robinson et al., (2013); Funston and Summers, (2013); Summers and Funston, (2013); Mossa et al., (2015); Du et al., (2017); Greenwood et al., (2017); and an excellent review from last year's ANAC conference by Moriel et al., (2018). The majority of previous research in developmental programming focuses around economically important traits like meat quality, growth and efficiency, and reproductive health. Figure 1 summarizes key timepoints throughout fetal development and the post-partum period in which nutritional intervention may alter phenotypic outcomes of progeny in beef cattle.

### *Growth and Feed Efficiency*

In Figure 1 traits such as placental development, organogenesis, and development of the uterus and testis occur during the first trimester of pregnancy which all can be impacted by nutritional intervention (Funston and Summers, 2013). In particular, nutrient restriction during early gestation has been shown to reduce uterine blood flow and can impact calf birth weight if nutritional stress persists (Long et al., 2009). The influence of early gestation on developmental programming events and vascular development are reviewed by Vonnahme and Lemley, (2012) and Reynolds and Redmer, (1995). Development of the fetal limbs and organs begins at approximately d 25 post-conception, where a sequential development of pancreas, liver, adrenals, lungs, thyroid, spleen, brain, thymus, and kidney along with heart and gastro-intestinal tract (Funston and Summers, 2013). Although some variation in results is reported, generally nutrient restriction impacts not only organ size but also vascularity (Meyer et al., 2010). This may have potential impacts on offspring growth, maintenance energy requirement, and nutrient absorption.

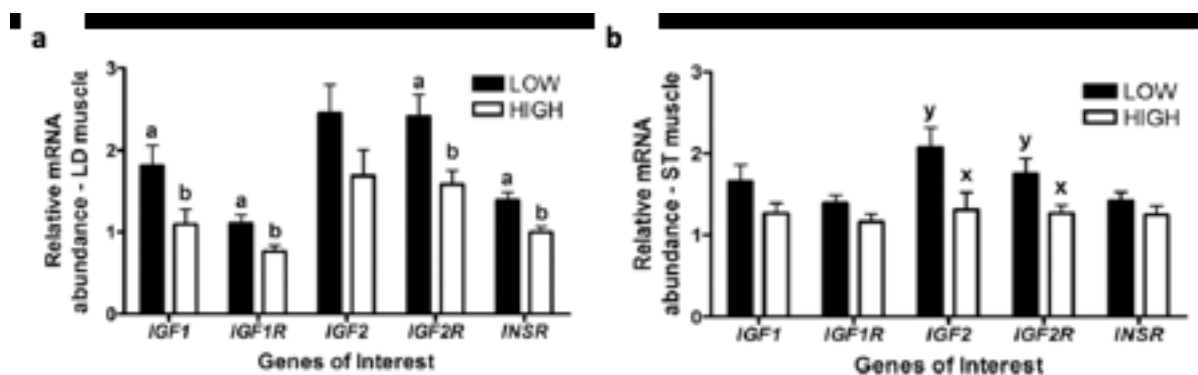


**Figure 1.** Key developmental timeline for economically relevant traits such as meat quality, reproductive development, growth and efficiency and their use as developmental programming in beef cattle. Partially adapted from Du et al., (2010)

Following early-to-mid gestational development of organs and the placentome, development surrounding growth of muscle and adipose tissue occurs and may have implications on post-natal growth and feed efficiency. The research in this area is largely based on the concept of the “Thrifty Phenotype Hypothesis” coined by British epidemiologist David Barker and based on increased metabolic disease states in people born following severe nutritional restriction during mid-to-late pregnancy (Hales and Barker, 1992). However, for the beef industry where increased fat deposition and low metabolic rate are desirable characteristics, these mechanisms have potential as a new management strategy to improve economically relevant traits like fat deposition and feed efficiency. This hypothesis describes a mismatch between the nutritionally restricted *in utero* environment and the affluent nutritional environment in which offspring are raised. In essence, this mismatch primes the genetics of the fetus to expect a nutrient limiting environment and following parturition the offspring does not encounter such hardships, resulting in improved metabolic efficiencies.

Although many of the mechanisms controlling this phenomenon are not well understood, research investigating insulin-like growth factor gene families are known to have implications on muscle growth both pre- and postnatally (Brameld et al., 2000; Costello, et al., 2008) and are a key target of developmental programming research in cattle. Micke et al., (2011) reported differences in IGF-1 and 2 which were accompanied by increases cross-sectional area of *longissimus dorsi* and

*semitendinosus* in mature male offspring born from dams restricted in protein in the first trimester and increased IGF-2 expression when exposed to protein restriction in second trimester. These researchers also noted an interaction with offspring sex which may also alter results. Wang et al., (2015) also noted changes in IGF-2 receptor in dams supplemented with a high grain diet during second trimester, further indicating that insulin-like growth factor may be a key target for developmental programming modifications. Previous work by our group found that nutrient restriction does not need to be severe in order to obtain fetal differences consistent with the thrifty phenotype hypothesis (Paradis et al., 2017). In a group of cows fed either free-choice (140% of total energy requirements) or restricted (85% of total requirements) from about d 150 of gestation to about d 250 of gestation, fetal tissue from *longissimus dorsi* or *semitendinosus* had mRNA expression differences in insulin-like growth factor related genes consistent with the theory of nutritional miss-match (Figure 2). In addition, diet impacted methylation of regions of IGF-2 (DMR2) and feed restriction reduced expression of micro RNAs associated with IGF-2 (miR-1, miR-133a) in *longissimus dorsi* tissue, suggesting that these genes are responsive to maternal nutritional intervention. Future research should continue to investigate the IGF family of genes as they appear to be promising targets for development programming and are also tied to economically important traits like muscle growth.



**Figure 2.** mRNA abundance of genes associated with insulin-like growth factors in *longissimus dorsi* (a) and *semitendinosus* (b) tissue isolated from fetuses from dams fed approximately 85% (low) or 140% (high) of total energy requirements from about d 150 to d 250 of gestation. Bars differing in letters differ  $P < 0.05$  (a,b) or  $P < 0.1$  (x,y). Adapted from Paradis et al., (2017)

### Meat Quality

Along with growth and efficiency traits, there has been significant advancement in the understanding of the impact of developmental programming on meat quality. Largely meat quality focuses on differences in muscle development and differentiation (lean growth) and adipogenesis (intramuscular fat or marbling). In general, muscle fibre hyperplastic growth occurs in early-to-mid

pregnancy, where primary muscle fibre growth occurs until about the second month of gestation and secondary muscle fibre hyperplastic growth occurring until approximately 7 months of gestation (Du et al., 2010; Moriel et al., 2018; Figure 1). Hypertrophic growth begins in mid pregnancy and continues past parturition until slaughter. Since other aspects of fetal development (organogenesis, nervous, skeletal) take priority over muscle growth, muscle development is highly vulnerable to nutritional stressors during gestation (Zhu et al., 2006). Aspects of developmental programming influence on muscle growth are well reviewed in Du et al., (2010); Robinson et al., (2013); Du et al., (2015); Du et al., (2017). Protein supplementation during mid-to-late gestation in particular has been shown to impact muscle fibre development. For example, protein supplementation (12% vs 6%, dietary CP) in mid-to-late gestation increased finished offspring rib-eye area, lean yield, and improved meat tenderness, despite no differences in collagen content or muscle fibre diameter, intermuscular fat or post-partum growth rate (Maresca et al., 2019). These studies suggest that mid-to late gestation nitrogen metabolism impacts muscle fibre hypertrophy, however the mechanism in which developmental programming controls this response is unknown.

In addition to muscle development, there is an increasing body of evidence suggesting that mid-to-late gestation nutrition has impacts carcass characteristics like marbling and yield grade. Adipogenesis commences in mid-to-late gestation, and adipocyte hypertrophic growth continues until slaughter (Du et al., 2015; Figure 1). Developmental programming studies suggest supplemental protein in mid-to-late gestation also improves lipogenic carcass traits in beef cattle as reported by Larson et al., (2009); Underwood et al., (2010); Shoup et al., (2015); Summers et al., (2015); Wilson et al., (2016) and reviewed by Ladeira et al., (2018). Although there are a variety of mechanisms which may be involved in the developmental programming response, peroxisome proliferator-activated receptor gamma, a nuclear receptor which plays a role in adipogenesis and cellular lipid uptake and known to increase intramuscular fat (Baik et al., 2017). Differences in PPARG mRNA expression were reported in fetal longissimus dorsi from restricted fed cows in late gestation (Duarte et al., 2014; Paradis et al., 2017;). A time by treatment effect was reported from birth to weaning of heifer born from cows fed at or 140% of protein requirements 9 weeks prepartum, where PPARG expression increased significantly more than heifers born from cows fed at requirements (Hare et al., 2019).

## **Reproduction**

Reproductive efficiency is perhaps one of the most important factors in profitability in the cow/calf production system, as it directly impacts annual net calf crop and indirectly determines cow longevity, as reproductive failure is the primary reason for culling cows from the herd. In general, key developmental periods which may impact reproductive efficiency occur in early-to-mid gestation (Figure 1). During fetal development, primordial follicle assembly occurs from d 80 until approximately d 150 of gestation, where the entire oocyte reserve is established for the reproductive lifespan of the developing heifer (Funston and Summers, 2013). Despite development of the reproductive tract in mid gestation, protein supplementation in late gestation has been shown to improve female progeny pregnancy rates and were more likely to calve in the first 21 days of the calving season (Martin et al., 2007). However, these may be as a result of improved growth rate, as heifer progeny were heavier throughout the trial. Roberts et al., (2016) reported similar results in protein supplemented range conditions during mid-gestation and a trend for longer productive life

from daughters of cows grazing improved pastures during 5-6 months of gestation. Although much more research is needed to better characterize the implication of developmental programming on reproductive performance and cow longevity, these results indicate that reproductive performance can also be impacted by nutritional intervention during gestation.

## Post-Partum and Lactocrine Programming

One novel research direction which may have strong implications for the beef industry is developmental programming responses surrounding the pre-and post-partum period. In the North American beef production system, often cows may be more intensively managed prior to calving, which may provide a practically feasible window for nutritional intervention and targeted developmental programming for producers. Although pre-partum implications of developmental programming include muscle hypertrophy and adipogenesis as illustrated in Figure 1, the impact of maternal nutrition on colostrogenesis and lactocrine programming is not as well researched in ruminants. Similar to the theory of the ‘thrifty phenotype’, the concept of lactocrine programming relays on a similar mismatch between in *utero* predicted environment and the actual postnatal environment which may cause metabolic dysregulation and altered growth.

Although colostrum is more often studied for its implications on passive immune transfer and subsequent calf health, colostrum also contains numerous bioactive components which may have implications for calf growth. This include a variety of proteins, lipids, carbohydrates, vitamins, and minerals. Many of these have functions for not only immune development, but a variety of hormones and growth factors (Tacoma et al., 2017). Table 1 illustrates the changes composition and bioactive concentrations in dairy cattle colostrum, and the decreasing concentrations over the first week and mature milk production. Although this table illustrates the shifting composition as colostrum matures into milk, less known is on how pre-partum maternal nutrition impacts the profile of the colostrum and abundance of other bioactives in colostrum.

Work from our lab (Radford, et al., 2018) investigated shifts in the proteome of colostrum of primiparous crossbred Hereford heifers fed isocaloric diets either 100% of predicted metabolizable protein (MP) requirements (n=7; CNCPS) or 133% predicted metabolizable protein requirements (n=6) for  $55 \pm 3$  d prior to parturition. Analysis of colostrum identified 213 distinct proteins, of which 11 were enriched and 13 were depleted in cows fed a high MP diet vs controls. Enriched colostrum proteins significantly associated with gut and immune system development ( $5.48\text{E-}08 < P < 2.49\text{E-}4$ ) and depleted colostrum proteins were significantly associated with growth regulation ( $5.48\text{E-}08 < P < 2.49\text{E-}4$ ). In addition, serum samples from progeny 6 h post-colostrum consumption identified 179 distinct proteins, of which 60 were common with colostrum. In calf serum, maternal dietary protein treatment enriched 28 and depleted 19 proteins compared to progeny from control fed dams. These proteins were distributed across 27 interdependant interaction networks. However, generally maternal protein supplementation decreased generalized inflammatory proteins [(pro-inflammatory markers (Serpins, ITIHs, MST1, etc) and non-specific macrophage stimulation (CREBBP, MST1)) and promoted a precision response immune [increased adaptive immunity markers (IgJ, Ig-like proteins, CD5L, etc)] ( $7.94\text{E-}9 < P < 6.34\text{E-}4$ ). Protein supplementation decreased colostrum fat % ( $3.4$  vs  $7.0 \pm 0.8$ ;  $P=0.003$ ) and tended to decrease net energy content ( $1.4$  vs  $1.7 \pm 0.1$ ;  $P=0.052$ ) of colostrum (Hare et al., 2019). Progeny were followed until 112 d of age, however, no treatment differences in heifer calf performance was observed

(Hare et al., 2019). Although this study has a limited number of animals, it clearly demonstrates the major impact of pre-partum nutrition on colostrum composition and passive transfer of bioactive compounds can be impacted by maternal diet in beef cattle. Further research is needed to follow calves to slaughter or reproductive age to determine if these effects have long term developmental programming impacts on growth, performance, or animal health

**Table 1:** Macronutrient and bioactive compound composition of Holstein colostrum (milking 1), transition milk, and mature milk<sup>1</sup>

Component	Unit	Milking					Mature Milk <sup>a</sup>
		1	2	3	4	5/6	
Dry matter	g/L	245	290	160	155	153	122
Crude ash	g/L	18	10	10	8	8	7
Gross energy <sup>b</sup>	MJ/L	6	4.8	3.9	3.8	3.8	2.8
Crude fat	g/L	64	56	46	50	50	39
Nitrogen free extracts	g/L	25	40	42	43	46	49
Crude protein	g/L	133	85	62	54	48	32
Essential amino acids	mmol/L	390	230	190	140	115	ND <sup>e</sup>
Nonessential amino acids	mmol/L	490	290	240	170	140	ND <sup>e</sup>
Immunoglobulin G <sup>d</sup>	g/L	81	58	17	12	ND <sup>e</sup>	<2
Lactoferrin	g/L	1.84	0.86	0.46	0.36	ND <sup>e</sup>	ND <sup>e</sup>
Transferrin	g/L	0.55	0.44	0.39	0.21	ND <sup>e</sup>	ND <sup>e</sup>
γ- Glutamyltransferase	μkat/L	509	284	145	102	83	52
Alkaline phosphatase	μkat/L	19	8	3	2	1	4
Asparate aminotransferase	μkat/L	1.5	0.9	0.5	0.3	0.2	0.1
Tumour necrosis factor-α	μg/L	5	ND <sup>e</sup>	ND <sup>e</sup>	ND <sup>e</sup>	3	<2
Insulin	μg/L	65	35	16	8	7	1
Glucagon	μg/L	0.16	0.08	0.08	0.05	0.03	0.01
Prolactin	μg/L	280	180	150	120	ND <sup>e</sup>	15
Growth hormone	μg/L	1.4	0.5	<1	<1	<1	<1
Insulin-like growth factor-I	μg/L	310	195	105	62	49	<2
Insulin-like growth factor-II	μg/L	150	ND <sup>e</sup>	ND <sup>e</sup>	ND <sup>e</sup>	ND <sup>e</sup>	ND <sup>e</sup>

<sup>a</sup>Measured > 14 days after parturition

<sup>b</sup>Measured by bomb calorimetry.

<sup>c</sup>For content of individual amino acids, see Hammon and Blum (1999).

<sup>d</sup>For content of immunoglobulin A and M, see Vacher and Blum (1993).

<sup>e</sup>ND = Not determined.

<sup>1</sup>Adapted from Blum and Hammon (2000).

In contrast to postpartum milk yield, all studies that report colostrum characteristics in non-dairy ruminants have observed that excessive or restrictive nutrient intake during late gestation will impact the process of colostrogenesis by either reducing yield (Swanson et al. 2008; Meyer et al. 2011; McGovern et al. 2015), altering macronutrient composition (Meyer et al. 2011; Banchero et al. 2006; Hare et al. 2018a), or a combined effect of the two (Banchero et al. 2004; Banchero et al. 2006; Meyer et al. 2011). The potential to alter colostrum composition and yield may be more significant to program metabolic development than targeting milk production alone, as the neonatal period is arguably the most high-risk period of any mammal's lifespan. Colostrum consumption can increase glucose and insulin concentrations in neonatal calves regardless of counterparts consuming similar quantities and ratios of macronutrients (Steinhoff-Wagner et al. 2011). Colostrum also modulates glucose-insulin homeostasis to at least four days postnatal (Scheuer et al. 2006.; Steinhoff-Wagner et al. 2011) and has potent impacts on the neonatal glucose-insulin systemic axis (Schäff et al. 2014) and anabolism (Sadri et al. 2017). Neonatal glucose-insulin homeostasis is likely regulated by bioactive colostrum compounds (Blum and Hammon, 2000) in conjunction with the macronutrients (Steinhoff-Wagner et al. 2011) delivered to the small intestine. Since both the macronutrient content and bioactive fraction can be altered by late gestation nutrition, it is highly probable that mismatch can occur as early as the neonatal period and may continue to have impacts on offspring metabolism, impacting growth and efficiency (Allace et al., 2012).

Ongoing work from our group at the University of Guelph is investigating the impact of late gestational supply of protein and methionine on the impact on post-partum calf health, and subsequent growth performance (Collins et al., Leivre et al., Lawson et al., unpublished 2019). Cows were fed isocaloric diets at 90, 100, or 110% of total metabolizable protein requirements, with or without rumen protected methionine for 8 weeks before parturition. Preliminary results indicate that protein restricted cows lost BW ( $P=0.02$ ), however methionine supplementation did not improve weight gain ( $P0.07$ ) pre-partum. However, methionine supplementation reduced circulating concentrations of isoleucine, leucine, lysine, serine, threonine and valine, while increasing plasma glucose ( $P0.03$ ). Calf birth weight was not impacted by pre-partum dietary treatment ( $P0.31$ ). Interestingly, calf serum samples obtained on d 2 post-colostrum consumption indicated total protein and IgG concentrations were lower for calves born from methionine supplemented cows ( $P0.01$ ), while cows supplemented methionine also had lower ( $P0.04$ ) MUN concentrations in colostrum. Calves are currently undergoing a growth performance trial to determine developmental programming effects long-term. Although these preliminary results are somewhat unexpected, they do suggest that methionine supplementation may alter nitrogen partitioning in late-gestation cows and alter colostrogenesis, which further supports the lactogenic theory of developmental programming.

## **Challenges and Opportunities to Industry Adoption**

Although developmental programming represents a new field of research with significant implications for livestock production, there are some challenges need to be further researched as this technology matures. Firstly, the beef industry largely functions as a low-input/low-cost of production system, which heavily relies on extensive management systems to reduce production costs. This means that for the majority of the year pregnant cows are subject to year-to-year variations in weather, precipitation, and environmental stressors beyond the control of the

producer. This means that maintaining precision nutritional control from year-to-year which may be needed for some developmental programming systems can be a challenge to implement in a production setting. This is one reason why fetal programming and epigenetic (multigenerational) changes in beef production systems are difficult to study and why study results can greatly vary. For example, Beard et al., (2019) reported that observed developmental programming effects on heifer reproduction were highly correlated with in utero precipitation records over a 46-year timespan. In addition, vast differences in environmental conditions of developmental programming research efforts may also create regional specific responses to nutritional challenges. Cattle adapted to temperate climates may respond differently than those adapted to more arid-type environments. Therefore there is a need to validate developmental programming results across a variety of climates and conditions.

Method of imposing nutritional stress may also pose a practical challenge for cow/calf producers. Currently the most developmental programming research uses the environmental mis-match approach of the “Thrifty Phenotype Hypothesis” to induce nutritional stressors at various points during the gestational period. Although effective in inducing developmental programming changes in offspring, very few studies continue to evaluate the long-term effects on dam performance, recovery, and longevity. In some cases, severe nutrient restriction may have negative impacts on milk yield, weaning weights, cow performance, and reproductive success of the dam herself. Although with proper herd management these cows may be able to recover, the combined developmental programming effects of these long-term management strategies have not been evaluated. Care should be taken in suggesting severe nutrient restriction as a technique to impose a fetal programming response to producers without considering the impact on dam longevity.

There is a body of research investigating protein supplementation strategies for beef cows and subsequent developmental programming effects on growth reproduction and other traits, of which a few were described above. However, many of these supplementation strategies may actually be correcting a nutrient deficiency, rather than looking at supply of nutrients relative to animal requirements. This may be particularly true of studies which have investigated grazing native or dormant ranges, where crude protein is often limiting, and cattle may be below nutrient requirements. In addition, this strategy makes it difficult to determine if developmental programming responses are due to supplied nitrogen or additional energy, as rarely are treatments compared on an isocaloric or isonitrogenous basis. More research is needed to more clarify if developmental programming responses are truly a response to supplementation or correcting a nutrient deficiency.

Another potential barrier to the implementation of programmed nutrition strategies may be due to the inherent segmented nature of the beef supply chain, and ultimately the transfer of economic value of the added costs of developmental programming up the supply chain. As a developmental programming approach largely relies on management during pregnancy and the post-natal period, the costs are incurred by the cow/calf producer. Although there may be some developmental programming systems with direct economic benefit to the cow/calf producer (reproductive efficiency, potential for increased weaning weights), the majority of economic gains are likely to be garnered in the feedlot (increased carcass value, improved feed efficiency). Therefore, just like many other management protocols which occur on cow/calf operations (pre-conditioning programs, vaccine protocols, low-stress weaning, etc) cow/calf developmental programming management protocols would need to have economic value. At this juncture, with challenges with

variable results discussed above, the economic value of these programs may be difficult to estimate at this time, however with more research and the development of standardized protocols this may present another management tool for producers. As the beef industry becomes more vertically integrated via increased numbers of retained ownership cattle on feed, stalker calves purchased on contract, and the popularity of calf-clubs to market feeder-calves, “programmed” cattle may be another common place management intervention to increase calf value.

As research continues to progress in understanding of the implications of developmental programming on animal performance and health, it is possible that nutritional programming protocols are developed to mitigate the impacts of nutritional stressors during pregnancy, multi-generational feeding programs, or for targeting outcomes for economically relevant traits. This represents a major shift the traditional approach to feeding beef cattle, however this approach may provide a new tool for producers to use to continue progress in improving beef cattle efficiency, health, and sustainable production.

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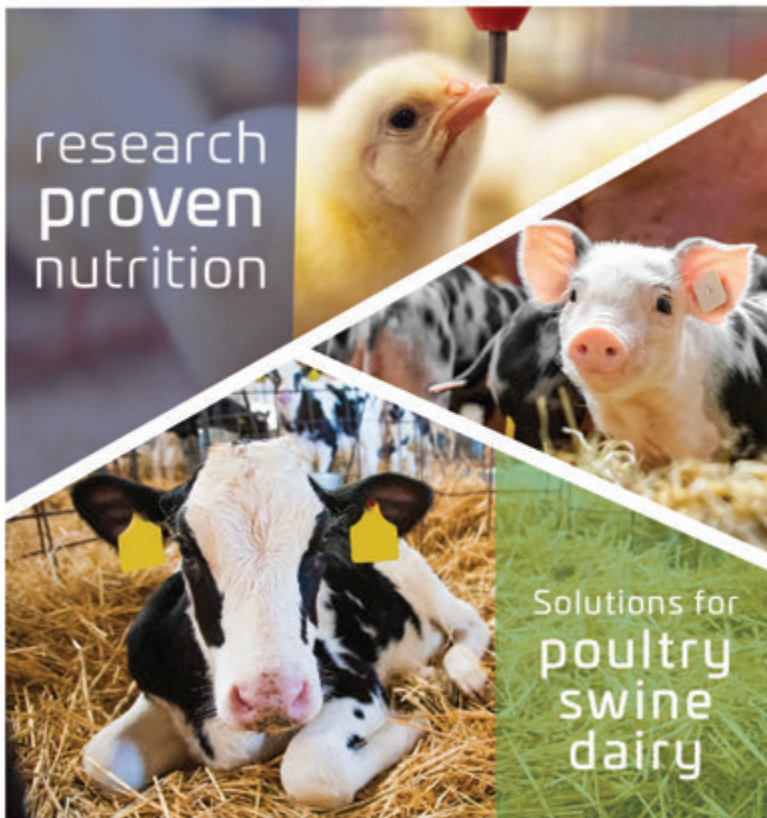
  
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## **Embryonic Response to High Beta-Hydroxybutyrate (BHB) Levels in Postpartum Dairy Cows**

### **Réponse embryonnaire à des niveaux élevés en bêta-hydroxybutyrate (BHB) chez la vache laitière en début lactation**

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#### **Abstract**

The increasing demand of milk production in the early lactation results in an important modification of the cow's metabolism, especially with its impossibility to fill this energetic demand only by feeding. This metabolic change forces the cow to tap into these reserves and subsequently leads to the production of ketone bodies such as  $\beta$ -hydroxybutyrate (BHB). This metabolite's production in the blood is then an important clue of the depth of the deficit in which the animal is. The embryos produced (D7 of embryonic development) under a characteristic level of BHB have been analysed (transcriptomic and epigenetic), in order to determine the impact of this critic period on embryonic quality. The results show that the most solicited genes are implied in specific pathways of transcriptional and mitochondrial regulation as well as energetic metabolism. It seems clear that the energetic deficit has an impact on the embryonic quality by altering, among others, the mechanisms implied in the energetic signalization at a cellular level and influencing the DNA's methylation. Although subtle, these modifications might have a considering impact on the animal's capacity to capture and retain the energy in adulthood.

#### **Résumé**

La demande accrue occasionnée par la production laitière en début de lactation entraîne une modification importante du métabolisme chez la vache, notamment par son impossibilité à combler cette demande énergétique par l'alimentation seule. Cette modification au niveau métabolique force la vache à puiser dans ces réserves et entraîne subséquemment la production de corps cétoniques comme le  $\beta$ -hydroxybutyrate (BHB). La production de ce métabolite au niveau sanguin est ainsi un important indice de la profondeur du déficit dans lequel l'animal se trouve. Les embryons produits (J7 du développement embryonnaire) sous un niveau caractéristique de BHB ont été analysés (transcriptomique et épigénétique), dans le but de déterminer l'impact de cette période caractéristique sur la qualité embryonnaire. Les résultats montrent que les gènes les plus sollicités sont impliqués dans des voies spécifiques de régulation transcriptionnelle, mitochondriale ainsi que du métabolisme énergétique. Il semble clair que le déficit énergétique a un impact sur la qualité embryonnaire en altérant entre autres les mécanismes impliqués dans la signalisation

énergétique au niveau cellulaire et en influençant la méthylation de l'ADN. Bien que subtiles, ces modifications pourraient avoir un impact considérable sur la capacité de l'animal à capter et retenir l'énergie à l'âge adulte.

## Introduction

Cow fertility has become a major challenge for the milk industry. Despite the fact that the milk production relies on the capacity to produce a calf the reproductive performance of Holstein cows has decreased considerably during recent decades (Casida, 1961; Butler, 1998; Lucy, 2001; Walsh *et al.*, 2011). This is regarded as a complex multifactorial problem (Butler, 1998; Veerkamp *et al.*, 2003) with interactions that make it difficult to determine a specific cause (Walsh *et al.*, 2011). Nonetheless, this decrease seems mostly associated with an intensive selection of production traits creating a metabolic deficit in early lactation. Indeed, after calving, cows undergo many physiological changes in response to the higher demand for milk. This often creates a negative energy balance, a common nutritional problem in lactating dairy cows and plausible cause of delayed recovery of fertility (Beam and Butler, 1999; Butler, 2000; Butler, 2001) due to carryover effects on the reproductive tract (Britt, 1992; Snijders *et al.*, 2000; Kruip *et al.*, 2001). Collective results indicate a detrimental effect of this period on oocyte competence for embryo development even though the metabolic effects may be not limited to follicular development (Butler, 2003).

During their development, cumulus-oocyte complexes and early embryos undergo multiple physiological changes and exhibit unique combinations of metabolic plasticity and sensitivity using a variety of metabolic substrates via multiple pathways. Although these cells are capable of adapting their metabolic activity to use a variety of nutrients in their environment, such adaptation often comes at a cost of reduced viability (Herrick *et al.*, 2017), functional alterations or altered metabolism (Cagnone *et al.*, 2012; Cagnone and Sirard, 2013; Cagnone and Sirard, 2014; Girard *et al.*, 2015; Laskowski *et al.*, 2017). Also, many rodent studies show that the impact of the metabolic environment during embryos development and lack of nutrients during pregnancy has been associated with an increased risk of suffering metabolic diseases in adult life. In cattle, several studies support the existence of embryonic programming during gestation that is characteristic of the environment in which the embryo evolves, leading in particular to a decline in fertility parameters in adulthood, as well as production and longevity of the animal in the herd (Mossa *et al.*, 2009; Evans *et al.*, 2012; Gonzalez-Recio *et al.*, 2012).

## Methodology

The experiment was conducted with Holstein cows (*Bos Taurus*; n = 18) at the Centre de Recherche en Sciences Animales de Deschambault (CRSAD) in Quebec, Canada. Cows were selected based on a 45-day postpartum  $\beta$ -hydroxybutyrate blood test value, which is a good indicator of the energy deficit depth. Based on that measure, the energy deficits were defined as high (BHB  $\geq 0.9$  mmol/L) or low (BHB  $< 0.9$  mmol/L). The cows underwent ovarian superovulation between days 8 and 14 of the estrous cycle, around 60 postpartum, to prepare them for insemination with sexed semen five days later. After a developmental period of 7 days, embryos were harvested, scored on a quality scale and mounted in straw, either individually or in groups.

Transcriptomic and epigenetic analyses were performed on embryos from 10 cows with an average of  $2.8 \pm 1.2$  lactations. A total of five morulae and one early blastocyst of good quality (I and II on a scale of I to IV) were selected for each biological replicate and pooled. Four biological replicates were produced for each of the two treatments. In the low BHB group, morulae were obtained from four cows and blastocysts from one cow. In the high BHB group, morulae were obtained from five cows and blastocysts from two cows.

## Results and discussion

### *Cow reproductive performance*

The BHB level had no significant relationship to the number or the quality of the embryos harvested. However, the higher proportion of morulae ( $p = 0.0295$ ) and consequently lower proportion of blastocysts might indicate a delay in the embryonic development during the first days of gestation in cows adjusting to a greater energy deficit, although the difference is slight. These results are consistent with what was shown *in vitro* with embryos produced from oocytes obtained from cows with higher levels of BHB in the follicular fluid (Sarentonglag *et al.*, 2013).

### *Impact on the embryos transcriptome*

The analysis of the relative expression of genes identified more than 1000 genes affected by the mother's signature. Of these, 823 were down-regulated in embryos from cows in metabolic deficit and 335 were up-regulated. The analysis of the most influenced pathways shows the modified expressed genes within the three principal categories, which are altered energy metabolism (mTOR and sirtuins pathway), mitochondrial dysfunction (oxydative phosphorylation) and inhibition of transcription (EIF2 signaling, regulation of EIF4 and p70S6K signaling). It appears that the presence of the metabolic deficit during embryonic development force the embryo to adapt to low energy through several mechanisms, in particular by altering the mitochondrial functions where several factors are involved. One of these factor, the mTOR pathway, seems to be particularly involved within this regulation.

The mTOR pathway is an important regulator of cell size that coordinates the activity of the cell growth machinery with the levels of energy and nutrients, such as lipids, amino acids and glucose (Sengupta *et al.*, 2010; Zoncu *et al.*, 2011; Kato and Perl, 2016). One of the complexes, mTORC1, acts as a sensor in many crucial pathways at the cellular level, like nucleotide biosynthesis, lipogenesis, glycolysis and autophagy (Ganley *et al.*, 2009; Hosokawa *et al.*, 2009; Ma and Blenis, 2009; Peterson *et al.*, 2011; Ben-Sahra *et al.*, 2013; Robitaille *et al.*, 2013). Autophagy is an intracellular recycling system, which constitutes an adaptive response to different kinds of stress by which the cells avoid cell death (Maiuri *et al.*, 2007). This mechanism is implicated especially in homeostatic function by removing aged cells, long-lived proteins and supernumerary or damaged organelle, but can also be induced by a change of environmental conditions such as nutrient depletion (Shintani and Klionsky, 2004). In energy-depleted states, elevated AMP (low ATP) levels activate AMP kinase and sirtuin 1 (SIRT1), which inhibits mTORC1 through phosphorylation of both the regulatory-associated protein of mTOR (RAPTOR) and the tuberous sclerosis complex 1 (TSC1) (Inoki *et al.*, 2003; Gwinn *et al.*, 2008; Inoki *et al.*, 2012). AMP-activated protein kinase also activates forkhead Box O (FoxO) transcription factors, increasing the expression of genes involved in stress resistance and energy balance (Chiacchiera and Simone,

2010). Inhibition of the first complex allows activation of the second complex (mTORC2), which is recruited to mitochondria-associated endoplasmic reticulum (ER) membranes (MAM), where it is known to interact with the mitochondrion and influence its functions. One of its specific substrates, serum- and glucocorticoid-inducible kinase 1 (SGK1), is located primarily in the mitochondrion outer membrane and could play a role in the regulation of this function. By altering the oxidative capacity, overproduction of reactive oxygen species (ROS) can alter the mTOR pathway and determine cell fate by promoting senescence. Another important substrate present in the ER is protein kinase B, also called Akt. Its presence in the ER suggests that, in period of stress, the mTORC2 could interfere with the Akt to regulate its functions. The mTORC2 complex can also be activated via a phosphorylation cascade triggered by the phosphoinositide 3-kinase (PI3K) pathway, which is a key regulator of survival during cellular stress. Other actors such as tumor protein p53 are also involved in the regulation of the mTOR pathway and the mitochondria under specific stress conditions

By its influence on the growth, as well as its interaction with the mitochondrion, the mTOR signalisation way is a key way expressed in the embryo when it undergoes the first development stages in an energy restrained environment. This adaptation by the embryo is translated by its metabolism slowing down and the use of an alternative method to use the energy.

### *Epigenetic signature*

The confirmation of these changes on the epigenome and their possible impact on the next generation has been done by measuring the DNA's methylation (450 000 cytosines) via the use of microarray, where the analysis allowed to determine 462 differentially methylated regions. Among these regions, 278 were more methylated in the high BHB group while 184 were more methylated in the low BHB group. Furthermore, 206 were associated with a gene or a presumed gene region. These sequences, spread across the genome, shown a methylation preference among all regions except for exons, which seemed to be more methylated in the control group. The analysis of the CpG content also showed some preferential methylation. CpG islands were more methylated in control group embryos, while other regions like CpG shores, CpG shelf and open sea were more methylated in BHB-stressed embryo genomes. These data suggest that the methylation status seems to allow the embryo to respond to the stressful environment in order to adapt itself. By promoting some characteristic pathways and enhance gene transcription of specific genes, the embryo may be able to adapt in an environment with high BHB level. Indeed, the analysis of the signaling pathways show interesting concordance with the transcriptomic analysis. The signaling pathways identified are involved in energy regulation (AMPK signaling and mTOR signaling pathway), cellular development (netrin signaling), inflammation response (leukocyte extravasation signaling) and stress response (GP6 signaling pathway).

Analysis of probe location revealed decreased methylation at sub-telomeric regions in every chromosome. This phenomenon, although surprising, was also observed in our laboratory on embryos that had received glucose supplementation during their early developmental stages (Tremblay *et al.*, 2018). The loss of methylation in these regions is associated with various types of cancer (Fraga *et al.*, 2005) and seems to produce a less compact and therefore more transcription-permissive structure. This suggests that the embryos may display a Warburg effect mimicking cancer-like methylation in order to survive, as has been shown in bovine and porcine embryos *in vitro* (Cagnone *et al.*, 2012; Krisher and Prather, 2012; Redel *et al.*, 2012).

## Conclusion

The alteration of many important pathways such as the mTOR lets predict that the embryo tries to adapt to this environment unfavorable to its development. Furthermore, the apparition of distinct methylation marks on the genome sustains it is possible that the information known on the embryo, evolving in an environment rich in BHB, could persist until adult age and could subsequently be transmitted to the offspring. Even though it would not be the first time this type of phenomenon is described (Zamenhof *et al.*, 1971; Susser and Stein, 1994; Pinheiro *et al.*, 2008), it is however impossible to confirm this affirmation. Nonetheless this mechanism could potentially be proposed as a cause of the declining fertility in dairy cows. By perpetuating an alternative metabolic management, offspring's produced under these conditions could not only have an improved capacity to capture energy, but also show an even more pronounced deficit in early lactation. Given the impact of the energy balance on the recovery of the reproductive system, it would be possible to think that an animal metabolically programmed to be more sensitive to this phenomenon could show decreased fertility parameters when being compared with its peers. However, this phenomenon is yet to be confirmed using subsequent studies, and with a more important number of animals.

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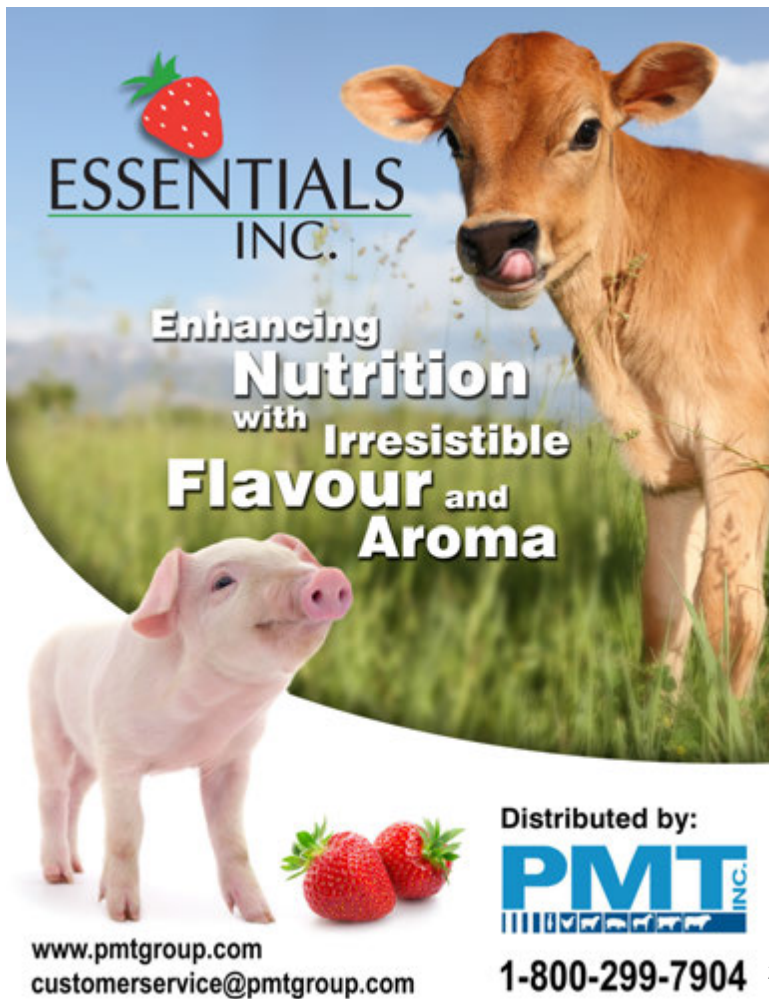


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## **“Epi-nutrigenomics”: Epigenetic Mechanisms as Links Between Nutrition and Performance in Livestock**

### **«L'épi-nutrigénomique» : mécanismes épigénétiques permettant de créer des liens entre la nutrition et la performance chez le bétail**

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#### **Abstract**

Nutrition is a key actor of health (metabolism, immunology and fertility) and performances in livestock but also in humans and animal models. An abundant literature describes the impact of various diets or of specific nutrients, whether restricted or in excess, on biological functions involving epigenetic mechanisms. Epigenetics are the molecular mechanisms responsible of genome function, modeling the chromatin structure and controlling the gene expression. Each of these mechanisms, namely DNA methylation and numerous posttranscriptional modifications of histone tails is submitted to specific dynamics for establishment, reading and erasure, all of which are ensured by active enzymatic processes. Metabolites and nutrients directly provide these enzymes and/or their co-factors. This review focuses on the central role of one-carbon metabolism, acetyl-CoA and NAD<sup>+</sup> on epigenetic mechanisms. Examples from studies in humans, rodent models and livestock animals will be used. Our objective is to analyze how nutrient status affects epigenetic marks, thus modifying multiple biological functions, taking a central place in the individual's fate and inducing inter-generational effect through fetal programming. Moreover, the relationship between host nutrition, rumen efficiency microbiota and epigenetic status will be explored. In the context of research on dietary changes in livestock animals in order to address climate changes and reduction of greenhouse gas emission by livestock, and taking into account food security in humans, research is needed to prevent negative effects of these new nutritional strategies on livestock health, performance, behavior and welfare.

#### **Résumé**

La nutrition est un facteur clé dont dépendent la santé (métabolisme, immunité et fertilité) et les performances que ce soit chez des animaux d'élevage ou chez l'Homme ou divers modèles animaux. Une littérature abondante décrit les effets de différents régimes ou de nutriments particuliers, que ce soit en excès ou de manière restrictive, sur les fonctions biologiques en impliquant des mécanismes épigénétiques. L'épigénétique est l'étude des mécanismes moléculaires responsables du fonctionnement du génome, participant à la structure de la chromatine et contrôlant l'expression des gènes. Chacun de ces mécanismes, à savoir la méthylation de l'ADN ou les modifications post-traductionnelles des histones, présente une dynamique spécifique pour son établissement,

sa lecture ou son effacement, impliquant des processus enzymatiques actifs. Métabolites et nutriments fournissent directement ces enzymes et/ou leurs cofacteurs. Cette revue cible plus particulièrement le rôle central du métabolisme mono-carboné, de l'acetyl CoA et du NAD<sup>+</sup> dans les mécanismes épigénétiques. Des exemples issus des études chez l'homme, les modèles rongeurs et chez les animaux d'élevage serviront d'illustrations. Notre objectif est d'analyser comment le statut nutritionnel affecte les marques épigénétiques, modifiant ainsi de multiples fonctions biologiques, pilotant le devenir de l'individu et pouvant induire des effets intergénérationnels via une programmation fœtale. De plus, la relation entre nutrition, efficacité du rumen, microbiote et statut épigénétique de l'hôte sera explorée. Dans un contexte où des évolutions des régimes des animaux d'élevage sont envisagées afin de répondre aux changements climatiques et à la réduction des gaz à effets de serre, et en tenant compte de la sécurité alimentaire de la population humaine, une intensification des recherches est nécessaire pour prévenir les effets négatifs de ces nouvelles stratégies sur la santé, les performances, le comportement et le bien-être des animaux d'élevage.

## Introduction

In all organisms, nutrition is a key factor to maintenance of cellular homeostasis. The impacts of nutrition on growth, health, fertility and performance were extensively studied in livestock (for bovine, see for review: Zebelli et al., 2015; Sordillo, 2016; Gelsinger et al., 2016; Baumgard et al., 2017; McGuffey, 2017; Rodney et al., 2018). Each physiological period (from the pre-conceptional period to ageing) and every physiological function requires a precise supply of specific nutrients in appropriate proportions. In order to cover the needs, it is important to better control the composition and quantity of nutrient supply in order to limit waste and improve nutrient efficiency. Alternatively, genetic selection offers the possibility to produce more efficient cattle. Another way could be to better determine the health/metabolic status of dairy cattle in order to better characterize their phenotype and their needs: in this respect, analyzing an individual's epigenome, as a representative marker of all life events, would provide useful information. Indeed, epigenetics is a concept originally coined by Conrad Hal Waddington in 1942 to describe the bridge between genotype and phenotype during development (Waddington, 2012). Subsequently, the definition shifted toward the notion of heritability: epigenetics concerns the perpetuation of gene expression and function across cell divisions without changes in DNA sequence. The epigenetic marks represent a memory necessary for propagating cell identity via the control of gene expression states. They can be modified by external environmental signals (including nutrition) and subsequently perpetuate altered transcriptional activity states even after the signal has disappeared.

Nutrition is one of the external signals with great variations and strong impact on epigenetics (Junien et al., 2006; Feil, 2006). The first clear demonstration of how maternal nutrition can influence the epigenetic regulation of gene expression in their offspring was reported using the

Agouti and Axin-fused inbred mouse lines (Morgan et al 1999). Indeed, maternal dietary supplementation with folic acid, vitamin B12, choline and betaine, led to clear shifts in offspring phenotype related to a concomitant increase of DNA methylation at the Avy locus (Waterland 2003).

A second elegant demonstration of direct interaction between nutrition and epigenetic process was reported in honeybees (*Apis mellifera*). In these species, two contrasting adult phenotypes are produced from the same diploid genome as a result of biological constraints. Two identical diploid embryos can develop either into a functionally sterile and short-lived female worker or into a highly reproductive and long-lived queen, depending on dietary intake (royal jelly or not) during postembryonic development. In 2008, Kucharski and collaborators demonstrated that the mechanism involved is DNA methylation. The silencing of the DNMT3A gene, coding for an enzyme required for de novo DNA methylation, in newly hatched larvae led to a “royal jelly-like effect” on the larval developmental trajectory with the majority of treated individuals emerging as queens with full ovary development and fertility. These data highlighted the fundamental interactions between Nutrition and Epigenetics and their biological consequences: distinct differences in bees’ behavior, physiology, longevity and reproductive capacity are driven by changes in the epigenetic landscape of specific transcriptional networks in response to early developmental diet

Clearly, nutritional status directly influences an individual’s phenotype but maternal and paternal nutrition can also have drastic consequences on offspring health (Aiken et al., 2016; Gabory et al., 2011). Convincing experimental evidence indicates that epigenetic marks serve as a memory of exposure to inappropriate environments in early life, and that these marks induce long-term changes in gene expression, potentially leading to disease in later life. The overall phenomenon is referred to as the Developmental Origins of Health and Disease (DOHaD; Barker et al., 1990), classically described for human health but clearly applicable for livestock. Maternal dietary effects vary depending on the period over which diet is applied, i.e., i) during the pre- and peri-conception period, through modification of oocyte quality and early embryo developmental competence (Dunford et al., 2017), ii) during gestation, through effects on foeto-placental development and iii) during the lactation period, where milk quality and/or quantity and maternal behavior may be disturbed. Recent studies have also shown that paternal dietary manipulation induces stable epigenetic alterations in male gametes that are sufficient to induce adverse metabolic phenotypes across generations of offspring (Fullstone et al., 2012; Dunford et al., 2017).

Since the 1980s, an increasing number of articles related to epigenetics have been published, with a marked bias towards studies in humans and mice and limited numbers in bovine, ovine and porcine species (Jammes et al., 2011; Singh et al., 2012; Funston et al., 2013). It became increasingly clear that epigenetic marks are key elements to fundamental developmental processes such as embryo genome activation, X chromosome inactivation and genomic imprinting, cell differentiation and organogenesis, DOHaD, ageing and onset of disease. A corpus of publications argues for a research strategy to better understand how epigenetics can provide a new framework to investigate complex traits in livestock (Jammes et al., 2011; Singh et al., 2012; Van Soest et al., 2014; Doherty et al., 2014; Naifeng Zhang, 2015; Ibeagha-Awemu et al., 2015; Chavatte-Palmer et al., 2016; Sinclair et al., 2016; Triantaphyllopoulos et al., 2016).

## **Epigenetic molecular processes: the state of the art**

Epigenetics is defined as the **molecular processes**, apposed on DNA molecules, that control chromatin architecture and gene expression without modification of nucleotide sequence . The epigenetic marks are mitotically, and sometimes meiotically, inherited and act as cell memory. All epigenetic marks are submitted to a dynamic cycle during embryo development, cell differentiation,

organogenesis and expression of biological functions. Each epigenetic mark's specific cycle is dependent on actors to pilot apposition and writing, reading and implication in genome functioning and finally, erasure. Active enzymatic processes and protein-protein interactions are involved. For each epigenetic mark, each step of the cycle represents a window of susceptibility to environmental changes that can induce alterations or modifications which subsequently are inherited by the next cell generation and transmitted to offspring as non-genetic information.

## **DNA methylation**

Evidences for methylation of nucleic acids dates back to more than 45 years ago: the first report was the presence of 5 methyl cytosine (5meC) in sea urchin embryos (Scarano, 1965). In 1975, Holliday and Pugh on the one hand and Riggs on the other described DNA methylation and gene activity during development.

DNA methylation occurs in almost all living organisms, from bacteria to plants and fungi, from invertebrates to vertebrates. Its abundance and role, however, highly varies across species. In vertebrates, and more precisely in mammals, DNA methylation occurs predominantly at the CpG dinucleotides symmetrically on the two DNA strands and is present in 5-10% of total cytosines of the genome. Non-CpG methylation, defined as methylation of cytosines within CpC, CpT and CpA contexts, is rare and only observed in embryonic stem cells (Ramsahoye et al., 2000), in male germ cells (Ichiyanagi et al., 2013), in induced pluripotent stem cells (iPSCs; Lister et al., 2011) and in the brain (Kinde et al., 2015).

Non-random distribution of CpG throughout the mammalian genome is observed. CpG rich regions, termed CpG islands, are defined algorithmically as sequences with an observed-to expected ratio of CpG greater than 0.6, a GC content greater than 0.5 and, in most cases, a length of more than 500 bp (Illingworth and Bird 2009). The methylation status of CpG islands is relatively stable across a variety of tissues and cell populations (Irizarry 2009). CpG islands are generally unmethylated when located within or near promoters or first exons of housekeeping genes. In contrast, when they are located in the promoter and regulatory regions of transposable elements, they are methylated, inhibiting the parasitic transposable and repetitive elements from replicating. The methylation status of regions along the CpG shores (<2kb flanking CpG islands) and CpG shelves (<2kb flanking outwards from a CpG shore) is reported to be more dynamic. The methylation status of regions with a lower CpG density than CpG islands can also have regulatory roles on gene expression depending on with the genomic context. A high methylation in close proximity to the transcriptional start site generally correlates strongly with gene expression inhibition whilst in distal regions (gene body and 3'UTR), it correlates more with an up regulation. It is also possible that gene body methylation may be highly correlated with alternative transcript isoforms.

In 2009, it was revealed that 5-hydroxymethylcytosine (5hmC) is another prominent cytosine modification catalyzed by the enzymes of the TET family and abundant in certain cell types

(Kriaucionis et al 2009). 5hmC has been thought to serve as an intermediate in the reaction of DNA demethylation or act as a signal for chromatin factors. Clearly, 5hmC is positively associated with gene expression (Mellen 2012) It is particularly present in the brain (around of 5%) and present in reduced proportion in others tissues (less of 0.5%).

## *Actors of DNA methylation*

**The writers.** DNA methyltransferases (DNMT) are enzymes that transfer a methyl group (CH<sub>3</sub>) to the cytosine of CpG dinucleotides. DNMT1 ensures DNA methylation maintenance and assumes the inheritance of epigenetic mark throughout mitosis. DNMT3A and DNMT3B are mainly devoted to de novo methylation and share a common cofactor, DNMT3L, a protein without DNA methyltransfer activity.

**The readers** of the DNA methylation are nuclear proteins with specific domains and properties to recognize the DNA methylation sites. They are members of the methyl CpG-binding domain (MBD) family (for example: MeCP2, MBD1-4). The MBD proteins can recruit histone deacetylases (HDACs) that contribute, with DNA methylation, to silence gene expression.

**The erasers.** The erasure of DNA methylation may be driven by one passive process and several active enzymatic processes. The passive process consists only in the absence of DNMT1 activity in the nucleus, leading to the loss of methylation by dilution after DNA replication; this process may be observed in the maternal pronucleus genome during the first phases of embryo development. Active processes include: i) a progressive oxidation of 5mC by TET enzymes and obtention of 5hydroxymethylated cytosine (5hmC) ii) action of DNA repair mechanisms with Base excision repair (BER) system or AID/APOBEC (Activation-induced deaminase / Apolipoprotein B mRNA-editing enzyme complex).

Two major epigenetic reprogramming events take place during early embryo development, soon after fertilization and in the germline. These reprogramming waves are accomplished by a massive loss of genome methylation.

- Soon after fertilization, an extensive chromatin remodeling occurs in paternal and maternal pronuclei in order to produce a highly decondensed DNA, to establish a chromatin landscape that will ensure the timely expression of developmental genes when the major zygotic genome activation takes place. The demethylation is passive in maternal pronucleus and active in paternal pronucleus. The global demethylation observed in the early embryo, however, excludes some genomic regions. Indeed, the imprinting center regions (ICR) are protected from this DNA methylation activity in order to assure the allele-specific gene expression of imprinted genes (Messerschmidt, 2012)

- The second wave of demethylation concerns the germline cells. The primordial germ cells (PGCs) are the embryonic precursors of mature gametes. First localized in the allantois (rodents) or in the yolk sack (ruminants), they migrate to colonize the gonadal ridges. During this period, PGCs undergo extensive reprogramming of their epigenome including a genome-wide DNA demethylation associated with histone modification. The subsequent apposition of epigenetic marks during gametogenesis is highly complex and different according to sex (figure 1)

## *Methods of DNA methylation analysis.*

Recent advances in epigenome engineering technologies now allow for the large scale assessment of the functional relevance of DNA methylation. Different technologies enable either the analysis of the global DNA methylation contents or the precise quantification of DNA methylation levels on single CpG positions. The study of DNA methylation may have a multitude of goals, including

understanding cellular and developmental plasticity in response to environment changes, mechanistic explanations for short and long term effects or finding new markers for diseases, pathologies or health status. Depending on the aim, it is important to take various factors into account such as the choice of an appropriate study design in relation with sample number and heterogeneity, the DNA quality, the choice of an appropriate analytical method, whether for a candidate-gene approach or a genome wide-approach. It is also essential to include validations and verification of results. A comprehensive overview of available technologies is reported in the third edition of “DNA methylation: Methods and Protocols” (Tost, 2018; figure 2). In this issue, a detailed description of technologies used is provided in order to better understand the advantages and limits in result interpretation. For example, native genomic DNA or bisulfite converted DNA can be used to study methylation. Moreover, several methods propose a global quantification of 5meC (HPLC, Elisa based upon antibody against 5meC use, etc). These methods do not take into account the non-random distribution of CpG in genome. Methods based on DNA cleavage using methyl sensitive enzymes enable the targeting of specific regions (CpG rich regions) as well as gene promoters (Luminometric Methyl Assay, LUMA; Karini et al., 2006; Perrier et al., 2018). Alternatively, it can be more interesting to quantify the methylation level at CpG sites. In this case, the sodium bisulfite conversion is considered the gold standard method, because epigenetic marks, methylated or unmethylated Cytosine (5meC/C), are converted in single nucleotide polymorphism C/T. In order to perform the quantification, it is possible to perform either a total pan genomic analysis (Whole genome bisulfite sequencing (WGBS), or to target part of the genome (Reduced Representation bisulfite sequencing (RRBS) or to perform a sequence specific analysis (pyrosequencing). Generally, the choice between technologies is commanded by biological questions and the funding available for the realization of project.

## Histone code

In the nucleus, microscopic analysis has long been used for the detection of nucleosomes (Oudet et al., 1975; Olins & Olins 2003). These structures, around which the DNA wraps (with a screw thread of 145–147 bp DNA), are formed by octamers of basic proteins, the called histones (2 x H2A, 2 x H2B, 2 x H3 and 2 x H4), that ensure the close compaction of DNA in the nucleus. The amino acid residues at the N-terminal tails of histones facilitate the addition of functional groups. Eight post-transcriptional modifications (PTM) have been described: e.g. acetylation of lysine, methylation of lysine or arginine, phosphorylation of serine or threonine, ubiquitylation, sumoylation, ADP ribosylation, deamination and proline isomerisation. Because these PTMs act at the level of different residues and at different position of the histone tails, at least 50 different modifications have been identified in relation with the target sites (Strahl and Allis 2000; Kouzarides 2007). The addition and removal of these groups are very flexible processes and have direct effects on the availability of the DNA sequence to the transcriptional machinery, affecting the activation or repression of gene expression (Kouzarides 2007). This large panel of PTMs acts with a combinatorial process amongst themselves and with other epigenetic marks such as DNA methylation and defines chromatin domains with various transcriptional activities. As for the DNA methylation, PTMs are dependent on epigenetic machinery including a large panel of proteins, with different roles such as writers, readers and erasers. Two different examples of major PTMs are explained here.

**Acetylation and deacetylation** is the most common post-translational modification of histones. The first report of acetylation as a post-translational modification was the acetylation of lysine residues on histone tails isolated from calf thymus nuclei (Allfrey et al., 1964). These modifications are transient and enzymatically controlled processes. Specific Histone acetyltransferases (HATs) can acetylate specific lysine residues in the N-terminus of all core histones. Histone lysine residue acetylation eliminates the positive charge of the histone, increases sterical hindrance and decreases the interaction of the N termini of histones with the negatively charged phosphate groups of DNA. The direct effect is correlated with active gene transcription and so-called open chromatin. This PTM can also bind proteins that contain acetyl-lysine recognition bromodomains (Mujtaba et al., 2007). Deacetylation, involving the four different classes of histone deacetyltransferases (HDACs), reverses this process, resulting in a closed chromatin structure (termed heterochromatin) that is transcriptionally repressed.

### ***Methylation and demethylation.***

Methylation is the addition of methyl groups to histones by enzymes called histone methyltransferases (HMTases). Methylation can occur at both lysine and arginine residues. Lysine residues can be mono-, di- and tri-methylated and arginine can accept two methyl groups (Jenuwein 2006). The addition of a methyl group can either activate or silence transcription. For example, di- or trimethylation of histone H3 at lysine 4 (H3K4) is associated with increased transcription, whereas di- or tri-methylation at lysine 9 and tri-methylation at lysine 27 is associated with transcriptional repression and results in heterochromatic states. However, the role of histone methylation depends on which amino acid is methylated and on the nearby presence of other methyl or acetyl group. For instance, acetylation of the tails of H3 and H4 along with the addition of three methyl groups on the lysine at position four of H3 (i.e., H3K4me3; remember that K is the abbreviation for lysine) is usually associated with actively transcribed chromatin. In contrast, a combined lack of acetylation of the H3 and H4 tails and methylation of the lysine in the ninth position of H3 (H3K9) is usually associated with highly repressed chromatin. Indeed, lysine methylations at H3K9, H3K27, and H4K20 are often associated with highly repressed chromatin. Histone demethylases mediate the removal of methyl groups from both arginine and lysine residues and contribute to reset of chromatin status.

### ***Methods of Histone PTMs analysis***

The Histone PTMs are widely studied. The genomic distribution of histone marks is commonly analyzed by immunoprecipitation (IP) of the chromatin using histone PTMs specific antibodies. The enrichment in IP product in comparison with in the input (genomic DNA without antibody interaction) can be analyzed at two different scales: a sequence specific scale by quantitative PCR (ChIP-qPCR) or high throughput sequencing (ChIP-seq). Currently, crosslinking is the most common technique to obtain IP products in human and rodent studies (ChIP-seq guidelines and practices of the Encode Consortia; Landt et al., 2012). This technique consists in a covalent fixation of the interactions between proteins and DNA using cross-linking reagents such as formaldehyde. Originally, crosslinking was used to improve the IP efficiency to study transcriptional factors – DNA interactions in order to fix the labile binding. In the case of histone PTMs analysis, the binding is considered much more stable and the use of native chromatin preparation is recommended to decrease the background noise (David et al. 2017). Clearly, better chromatin IP methods should be implemented in particular in animal livestock. The best method may vary depending on the tissue/ species/ PTMs studied.

## Small and long non coding RNAs

Since complete human genome sequencing in 2003, numerous sites of transcription associated with intron/exon structure of genes have been described and found separated by long stretches of intergenic space. The existence of a non-coding RNA population is now clearly established (Functional Annotation of the mammalian Genome, FANTOM project). Non-coding RNA refers to a large group of endogenous RNA molecules that have no protein coding capacity. Several classes are described based on their length, biogenesis, polarity (sense or antisense), and putative functions. A basic classification criterion is size

**The long ncRNAs (lncRNA)** are typically >200 nt-long, include long intergenic non-coding RNA, natural antisense transcript (NAT), transcribed ultra-conserved region (T-UCR) and non-coding pseudogenes. They function without major prior processing. The most famous lncRNA is Xist, that allows the X dosage compensation by epigenetic processes in mammalian females. Indeed, where males have only one X chromosome and females have two, dosage compensation requires inactivation of one female X chromosome in somatic cells. Substantial diversity in the timing and epigenetic regulation of X-Chromosome Inactivation initiation has been described in mammals (Okamoto et al., 2011). In human and rabbit embryos, Xist RNA appears firstly expressed from paternal and maternal X chromosomes, suggesting that Xist expression is not imprinted in early embryo developmental stages, in contrast to the paternal imprint of X observed in mice. In pigs, the same results as found in rabbits and humans were recently reported (Ramos-Ibeas et al., 2019) using RNA-seq at single cell resolution at different earlier embryo stages.

**The small non-coding RNAs** (< 200nt; **sncRNAs**) include small interfering RNA (siRNA), microRNA (miRNA), piwi-interacting RNA (piRNA) and microRNAs (miRNAs). Transfer RNAs, ribosomal RNAs, and small nuclear (sno) RNAs are also classified as sncRNAs. All are processed from longer precursors. They have a wide range of functions such as the regulation of gene transcription and translation, post-transcriptional modification, epigenetic landscape establishment, protein scaffolding and cofactors recruitment.

Eukaryotic sncRNAs are processed from longer precursor ncRNAs and share the common feature of serving as sequence-specific guides for Argonaute (AGO) proteins to regulate their targets. Their expression, biogenesis, and modes of action differ to various degrees. Among sncRNAs, miRNAs have been, by far, the most extensively studied. Briefly, miRNAs are 20–24 nt RNAs that are processed by Dicer enzymes from stem–loop precursors found in IGRs, introns, or coding regions. miRNAs use base pairing to guide RNA-induced silencing complexes (RISCs) to specific messages with fully or partly complementary sequences. The repression of targeted messages is a common outcome of RISC recruitment and might occur through translational inhibition, accelerated exonucleolytic mRNA decay or slicing in miRNA–mRNA pairs. Animals and plants express hundreds of miRNAs that are thought, at least in animals, to regulate a large part of the protein-coding transcriptome. miRNAs have vital roles in development, stress adaptation and hormone signaling.

The piRNAs (Piwi-Interacting RNA) are mostly involved in post transcriptional silencing of transposons, conferring them a role in safeguarding the germline from mobile DNA elements (Weick et al., 2014).

Transfer RNAs (tRNAs) are the most abundant small non-coding RNA molecules making up 4–10% of all cellular RNA. Their major role is to deliver amino acids to the ribosome to decode the genetic information on the mRNA for protein synthesis. Recently, using high-throughput sequencing, small non-coding RNA fragments (two classes of fragments based on the size: 14-30 nt and 30-40 nt in length, respectively) have been identified that are derived from tRNAs. These tRNA fragments display cell-type specific expression and have important functions, including the regulation of translation through mechanisms that are distinct from the role of mature tRNAs in amino acid delivery. A number of recent studies have highlighted that dietary manipulation in mammals can influence the expression or function of a number of classes of non-coding RNAs (Law et al., 2018).

Crosstalk between all epigenetic molecular processes, DNA methylation, Histone PTMs, Inc and sncRNAs enables the establishment of cell-specific epigenetic programming, regulates these processes during development and integrates environmental adversity. Thus, the epigenome may be considered as cell memory, representative of all past events during the life of an individual.

## **Nutrition and elements with epigenetic effects**

### *S adenosylmethionine (SAM), universal provider of methyl group*

All enzymes responsible for the methylation of DNA as well as that of histone arginine and lysine residues use SAM as the methyl group donor. SAM is synthesized directly from methionine, which synthesis is contingent on the “One carbon metabolism” (figure 3). The one carbon metabolism utilizes a variety of nutrients, such as glucose, vitamins, and amino acids. Two major components of one-carbon metabolism are folate and methionine, providing methyl groups for chromatin methylation through the generation of SAM.

The mammalian cells are unable to produce folate. They rely on external sources, thus rendering folate an essential nutrient. Folate is naturally present in several foods, including green leafy vegetables, fruits, cereals and animal liver products.

In monogastric animals such as pigs, at least 80% of folate requirements is supplied by food; the remaining 20% can be acquired through folate biosynthesis by intestinal bacteria present in colon (Asrar et al., 2005; Kok et al (2018)..

In ruminants, the landscape is quite different because the rumen bacterial populations can synthesize folate (for review see Ragaller et al., 2009). Moreover, the microbial activity, as well as the ruminal bacterial populations are influenced by the level of concentrates in the diet and by the type of feed. For example, high-concentrate diets result in an increase in available folate. As some bacterial species are able to synthesize folate, whereas others need them, the amount of folate that is finally available for the host’s “one carbon metabolism” is highly variable.

While clear beneficial effects of folate and/or vitamin B12 supplementation in dairy cows were reported as early as 1989 by Girard and collaborators, no analysis of chromatin methylation was associated. More recently, the effect of diet supplemented with rumen-protected D,L methionine during late-pregnancy and early lactation was reported. The supplemented diet directly affects the hepatic transcriptional activity in dairy cows, with a greater expression of genes associated

with the “one carbon metabolism” (*SAHH*, *MAT 1A*, *MTR*). Moreover, reduced expression of glutathione metabolism gene (*GSS* and *GCLC*) and *SOD1* reflects a lower oxidative stress and mild inflammatory status (Osorio et al., 2014). Moreover, methionine supplementation was shown to activate PPAR- $\alpha$  regulated signaling pathways associated with a global DNA methylation decrease but to induce a specific increase in methylation at the level of *PPAR- $\alpha$*  promoter region (Osorio et al., 2016). A similar maternal methionine supplemented diet has been reported to directly affect the calf hepatic metabolism with an increase of expression genes associated with methionine metabolism and DNA metabolism and trans-sulfuration (Jacometo et al., 2017). Batistel et al. (2019) also observed that maternal methionine supplementation during late pregnancy induces sex-specific divergent responses. At term, the maternal methionine supplemented diet increased the abundance of DNMT3A, a DNA methylation writer, only in female placenta but decreased the global methylation. Placental alterations in metabolic and epigenetic signatures were also observed. At birth, the body weight of male but not female calves born to cows receiving greater methionine supply was increased, without modification of global DNA methylation. Clearly, maternal rumen-protected methionine supplementation during late gestation could alter placental function and offspring postnatal growth but the underlying mechanisms are not established. Additional pan-genomic analysis is required to clarify the potential effect of methionine supply on the programming of offspring’s genome.

### **Nutrition and Histone acetylation / desacetylation, a balance between acetyl-CoA and NAD<sup>+</sup>/NADH ratio**

Acetyl-CoA is a key metabolic intermediate in both catabolic and anabolic processes. Beyond these metabolic transactions, acetyl-CoA serves as sole donor of the acetyl group for reactions catalyzed by acetyl-transferase such as histone acetyl transferases (Choudhary et al., 2014). Acetyl-CoA freely diffuses through the nuclear pore complex. Altering available acetyl-CoA in the cytoplasm or nucleus can cause changes in histone acetylation. In most mammalian cells, acetyl-CoA is predominantly generated in the mitochondrial matrix by various metabolic pathways, namely glycolysis,  $\beta$  oxidation and the catabolism of branched amino acids. Cytosolic acetyl-CoA, however, can also originate from glutamine reductive carboxylation when glycolysis is blocked or in hypoxic conditions, or be produced from acetate in an ATP-dependent manner. In response to a variety of physiological or pathological conditions, the abundance and/or the distribution of acetyl-CoA in the cytoplasm/nucleus compartments changes considerably. Thus acetyl CoA can act as a second messenger that relays signals from the extracellular to the intracellular milieu (Pietrocola et al., 2015). Acetyl-CoA being the compulsory cofactor for histone acetyltransferases (HATs), a drastic decrease in nucleo-cytosolic acetyl-CoA concentrations may directly reduce the enzymatic activity of HATs, supporting the epigenetic control of global transcription. Moreover changes in acetyl-CoA concentrations can alter the acetylation state of transcription factors, adding another regulatory level of gene expression.

Nicotinamide adenine dinucleotide (NAD<sup>+</sup>) metabolism NAD<sup>+</sup> is a cofactor used to carry electrons in numerous metabolic redox reactions and involved in processes including calcium signaling and protein deacetylation. The sirtuin family is a unique group of proteins with a NAD<sup>+</sup> dependent desacetylation activity, providing potentially important links between metabolism and epigenetic regulation of gene expression. The increase in the NAD<sup>+</sup>/NADH ratio observed during starvation promotes histone deacetylation by stimulating sirtuins’ activity (Guarante, 2013).

Finally, variations in intracellular pH have a major impact on histone acetylation: intracellular acidification (i.e. decreased of pH) promotes a desacetylation of histones whereas intracellular alkalinization (i.e. increased of pH) augments global histone acetylation. Of note the chromatin of mammalian cells contains at least 109 acetylation sites, meaning that massive histone acetylation/deacetylation may have major impact on transcriptional status and cellular metabolism.

## **Epigenetic effects of natural elements**

The nutritional environment is a major concern considering the current challenges faced by the livestock industry: environmental emissions, climate changes, accelerating food-feed-fuel competition for arable land. Recently, Halmemies-Beauchet-Filleau et al., (2018) reviewed the nutritive value of some current underutilized or novel feeds for ruminants. The effects of these feeds on ruminant milk production and quality as well as meat production were examined in comparison with performance obtained with conventional feeds. Nevertheless, natural compounds may have proven *in vitro* or *in vivo* effects on epigenetic machinery (see table 1). Although the use of more diverse and fibrous feeds not suitable for the nutrition of humans may benefit ruminant production, these information highlight the needing to perform long term evaluation of feed changes on the health, performance and welfare of ruminants.

For example, recommendations for reducing C footprint in dairy cattle include the use of maize silage, forage and rough feed. This diet highly rich in fiber, proposed in ruminants, reduce the proportion of methionine and lysine in the duodenum, that been considered the most limiting AA in North American diets for lactating cows (Lean et al., 2018). Clearly the decrease in methionine availability could have effects on DNA methylation and modify health, efficiency and performance of dairy cows and offsprings.

## **Nutri-Epigenomics: individual and inter-generational impacts**

As previously mentioned, there is an increasing interest to better define the epigenetic contribution to the phenotypic construction in livestock. To analyze the literature, different points of view are possible:

- To focus on cell- or tissue-target and function, i.e. describe epigenetics processes involved in cell differentiation-, organogenesis- and function (gametes and fertility, embryo and embryo development ability, mammary gland and lactation and milk production, muscle and meat quality and quantity).
- To analyze the windows of epigenetic reprogramming associated with susceptibility to alteration at different periods of life: peri-conception, gestation, post-partum, weaning, puberty, and aging. In dairy cattle, the need to stack lactation and gestation implies a focus on fetal programming and questions maternal transmission.

Recent studies also argue for paternal transmission of non-genetic information and offspring programming.

The interest of the first point of view is to highlight the major importance of epigenetic processes in the phenotypic construction and to raise awareness. The second point of view focuses attention on

the dynamics of these processes with an integration of effects at short and long terms. In this case, the major interest is to better define the critical developmental windows where adequate nutrition is essential. Here, the inter-generational effect may not only be considered for their negative effects but also as potential levers to promote positive programming and improve offspring health, behavior and performance. In this review, only a few examples will be given.

## **A focus on epigenetics in mammary gland development and function**

Mammary gland morphogenesis begins during embryonic development and proceeds postnatally through puberty, pregnancy, lactation and subsequent involution (Topper and Freeman 1980). The development of the mammary gland corresponds to periods of cell proliferation, cell differentiation and apoptosis in conjunction with changes in gene expression patterns. Epigenetic mechanisms, together with expression and activity of specific transcriptional factors, drive the spatio-temporal expression of genes responsible for cell fate determination. More precisely, epigenetic processes are involved in the regulation of lineage commitment within the mammary gland epithelium (Holliday et al., 2018). Indeed, in rodent models and in humans, studies have demonstrated the implication of DNA methylation, PTM of histones patterns and sncRNA catalog, i.e. all epigenetic mechanisms involved in maintenance and renewal of stem/progenitor epithelial cells, differentiation and function of luminal cells, and differentiation of myoepithelial cells (Holliday et al., 2018). In livestock, although a large body of literature addresses cellular and molecular function of the mammary gland (Akers 2017), studies describing the epigenetic machinery during mammary gland differentiation, development and activity remain scarce. Yet, it is obvious that during the development of the mammary gland as well as during subsequent lactation, farm management practices such as nutrition and milking frequency, photoperiod, pregnancy and diseases are well known to influence cell number and activity and milk production

### ***Epigenetic landscape controlling milk gene expression.***

The first demonstration of epigenetic and chromatin conformation modifications during normal mammary gland in lactating cows dates back to Platenburg et al., 1996. Lactation was associated with a hypomethylation of two CpG sites flanking the bovine alpha S1-casein gene and one in the 3'-region in mammary gland. In the liver, alpha S1-casein gene is not expressed and these CpGs are hypermethylated. Vanselow et al., further showed mastitis has such as consequence the hypermethylation of the same CpGs, correlated to decrease of milk production (2006). Singh and colleagues (2010) reported also the hypermethylation of these two CpGs closely related to involution. At contrast, Nguyen et al., 2014 reported that a progressive demethylation of these CpG positions occurs during gestation to reach hypomethylation at lactation. Altogether, these results demonstrate the setting up of specific epigenome landscape during normal mammary gland development and functional differentiation at milk gene promotor regions involving DNA methylation and histones PTM (Rijnkels et al., (2010); Rijnkels et al., 2013).

### ***Mammary stem cells, a target of environment changes.***

There is recent increasing interest focusing on mammary gland stem cells, based on the fact that parenchyme tissue development in early life as well as in the peripubertal period underlines the future mammary development and function (Akers 2017). Enhanced preweaning feeding in

the bovine appears to enhance the capacity of the mammary tissue to respond to mammary stimulation. This suggests that improved early nutrition might allow for the development of stem or progenitor cell populations that would better support the massive ductal growth and lobulo-alveolar expansion during gestation. The epigenetic landscapes of the different progenitor stem cells (mammary stem cells (MaSC), ductal-, alveolar- and luminal- progenitors) need to be further detailed in order to better understand i) the adverse impact of over-feeding in heifers during the first year after birth on milk production or ii) the positive effect of energy restriction during mid gestation, resulting in improved mammary development and subsequent lactation performance (Akers 2017).

### *Milk, as transporter of non-genetic inheritable information*

Milk is a central nutrient for the young during the early post-natal period and is important in human diet through the consumption of milk and derivative products. Nutritional quality of milk depends on its composition. The nutrition of the cow affects both milk production quality and quantity. Beyond the classical milk components, such as bioactive proteins, long-chain fatty acids, complex oligosaccharides, calcium, phosphorus, potassium, vitamin A, B2 and B12, milk also contains several components such as immunoglobulins for passive transfer of immunity (strongly associated with the colostral phase) and a plethora of sncRNAs. Among sncRNAs, catalogs of miRNAs expressed in the lactating mammary gland of ruminants were recently published (Mobuchon et al., 2015; Wicik et al., 2016) and the effects of dietary changes (Mobuchon et al., 2015 and 2017) or mastitis (by after *Staphylococcus aureus* infection; Li et al., 2015) were reported. These miRNAs are also identified in milk, not only in a free form but also packaged inside carrier vesicles (“exosomes”), suggesting a protection after milk ingestion that potentially could facilitate their absorption by the suckling young. Remarkably, the 14 most highly expressed miRNAs of bovine milk fractions are related with target genes associated with organism development (Ibeagha-Awemu et al., 2015). Thus, under physiological conditions, the transfer of milk-derived exosomes and their sncRNAs contents, operating exclusively during the postnatal period, may be considered as a major actor regulating offspring metabolic and immunological programming. This emerging research field highlights the importance of the control of sncRNAs synthesis by the mammary gland and of the packaging of microvesicles under various nutritional conditions in dairy cows. Further work in this area is required.

## **Focus on epigenetic programming, identification of susceptibility windows and offspring health programming**

### *Maternal nutrition and programming of offspring health and performance*

Maternal nutrition is one of the most important factors affecting fertility in cattle (Rodney et al., 2018). From all published studies, different periods appear to be crucial, with high sensitivity to environmental changes. These include the peri-conceptional period with oocyte and early embryo development, the peri-implantation period when uterine ability to enable embryo implantation is seminal, and the feto-placental period with contribution with placental development and function and in nutrient providing to foetus throughout gestation.

### *Pre-conceptional period: epigenetics of Oocyte.*

The links between maternal nutrition, oocyte metabolism and reproductive outcomes have recently been reviewed (Leroy et al., 2015). Oogenesis takes place during foetal life; at birth, the stock of oocytes included in primordial follicles is arrested at an early stage of the first meiosis division. Only after puberty, oestrous cycles are associated with oocyte and pre-antral follicle development in order to produce mature oocytes able to be fertilized. Studies in rodent models reported that the oocyte epigenome is dramatically remodeled during oogenesis. Global DNA methylation is low in early oogenesis, gradually increases and peaks as oocytes reach full size (see review Duffié and Bourc'his, 2013). Maternal imprints are established during oogenesis at selected imprint control regions (ICR) involved in the control of monoallelic expression of imprinted genes (most imprinted genes are hypermethylated on the maternal allele). The relative hypomethylation in the growing oocyte, however, is considered necessary for the maintenance of a high rate of transcription, necessary to produce and store the large quantity of maternal RNAs and proteins that are needed as maternal factors until embryonic genome activation. In fully-grown oocyte, DNA methylation induces a global transcriptional silencing of cellular genes. Histone methylation and acetylation are also regulated during oocyte growth and maturation. Acetylation of histone 3 (K9 and K18) and histone 4 (K5 and K12) increases as the oocyte nears ovulation and the chromatin rearranges into a nuclear configuration (Kageyama et al., 2007).

Maternal nutrition can directly affect the oocyte or effects can be mediated by the tight link between metabolism of both the oocyte and granulosa cells of follicle. In postpartum dairy cattle with a negative energy balance (NEB), the apposition of maternal imprint (methylation of ICR of *SNRPN*, *IGF2R*, *PLAG1*, and *PEG3*) in oocytes collected from follicles of greater than 3mm in Holstein-Friesian is more variable at 45-55 days postpartum than at 85-110 days postpartum (O'Doherty et al., 2014). This alteration outlines the effect of postpartum NEB on genomic imprinting and may contribute to early embryonic mortality, explaining the low fertility in postpartum dairy cows.

The oocyte epigenetic maturity is also questioned when maturation occurs *in vitro*. Exposure of oocytes to pathophysiological concentrations of Non-esterified fatty acids (NEFA) alters embryo developmental competence and influences overall DNA methylation patterns in the resultant blastocysts, affecting many genes (promotor or gene body) associated with cell death and survival and cellular metabolism. Aberrant DNA methylation patterns were observed in pathways related to caspase activation, p53-induced apoptosis, Ras-signalling and Wnt-signalling at the blastocyte stage (Desmet et al., 2016).

Oocyte vitrification is currently an emerging technique in cattle reproduction biotechnologies (International Embryo Transfer Society IETS 2016). Vitrification has an impact on subsequent embryonic development with epigenetic abnormalities reported at the blastocyst stage (DNA methylation; acH3K9; Chen et al., 2016).

The consequences of epigenetic maturity of oocyte are also due to inheritance of large domains labelled with specific histone modifications during oogenesis by the early embryo's genome and may be responsible for the control of early embryonic gene expression (Leese 2015).

### *Maternal metabolism, foetal programming and performances.*

In dairy cattle, based on an analysis of large national databases in the UK and in Ireland, it was shown that offspring from dams producing more milk before and during conception had reduced milk yields, increased somatic cell counts and were culled earlier compared with those born to dams with lower milk yields (Banos et al., 2007; Berry et al., 2008). Similar observations were made in Spain, where analysis of data in the Holstein breed showed that females born to dams that were lactating during early pregnancy produced significantly less milk compared with those born to dams that were not lactating, and that this reduction in milk production was correlated with maternal production (Gonzalez-Recio et al., 2012). However, no epigenetic analysis was associated and the molecular basis of this programming is lacking.

### *Paternal nutrition and offspring programming*

As for female gametes, the male gametes originate from a long cell differentiation process taking place during fetal life. In contrast to female gametes, it was reported that the spermatogonia pool displayed a quite full DNA methylation at birth; the acquisition of histone PTM, histone variants and the histone-protamine translation proper to spermatozoid being more related with subsequent steps of spermatogenesis. Recent studies demonstrated that DNA methylation is also dynamic at time of puberty, vulnerable to environmental changes during the reproductive life and altered with aging in human (Craig et al., 2017) as well as in bull (Lambert et al., 2018). The effects of diet on epigenome of human semen quality were reported: the expression level of specific miRNAs, piRNAs, tRFs, and small nuclear RNA (snRNA) fragments was altered in the spermatozoa from obese men (Donkin et al., 2016). Moreover, 9,081 unique genes have been found differentially methylated between lean and obese men. Interestingly, the gene list contains 274 genes identified as master regulators of appetite control including melanocortin-4 receptor (MC4R), brain-derived neurotrophic factor (BDNF), neuropeptide Y (NPY), cannabinoid receptor type 1 (CR1), CART, and genes related to obesity and metabolism. These results suggest a specific remodeling of epigenetic marks for genes controlling the function of the CNS and metabolism. Although a very large epigenetic reprogramming of paternal pro-nucleus occurs after fertilization, these genomic regions may conserve the marks and influence the gene expression in the post-fertilization embryo. Thus, epigenetic changes in gametes of obese men may influence the metabolic profile of their offspring.

### **Manipulation of maternal environment, as driver of epigenetic plasticity of offspring behaviour**

Using rodent models, the long term impacts of early environmental experiences on development have been explored extensively. These data corpus help to better understand the mechanisms mediating risks in human health such as psychopathology risk for individuals exposed to childhood adversity. In livestock, the ability of the animal to respond to its environment can affect the animal itself, but also the breeder, because loss of behavioral adaptation could lead to increased mortality, morbidity or affect ease of handling. In farm animals, a detailed mechanistic understanding of the effects of maternal or fetal prenatal stress is lacking.

A negative lever is classically the maternal stress. Some housing conditions and practices in livestock husbandry may be stressful for the animals and mother welfare problems could reduce vitality and well-being of the offspring. In pigs, Otten et al., (2015) reported several studies showing that fetal brain regions relating to Hypothalamo-Pituitary-Adrenal axis (HPA) function appear highly vulnerable to maternal stress with changes in postnatal life as a consequence of prenatal stress (PNS). Stress and feeding behaviors are regulated by the same key genes (CRH, POMC for instance) but the link between maternal stress and offspring feeding behavior, in terms of epigenetics, remains unknown.

More interestingly, the poultry domestication is associated with changes in morphology, physiology, behavior such as fearfulness (Nätt et al., 2012). The comparison between domesticated White Leghorn layers and their wild ancestors, the Red Junglefowl revealed differential methylation and gene expression in hypothalamus/thalamus. The epigenetic states may have been an important mechanism involved in the rapid evolutionary changes of chickens during domestication in particular in behavior changes.

Studies concerning the link between maternal nutrition and offspring behavior with a special focus on epigenetic mechanisms are scarce, and mainly concern rodents or human. In these studies, it is nonetheless difficult to conclude on a direct or indirect effect of the diet (altered maternal behavior, inflammation). For example, a maternal high fat (HF) diet induced an increased preference for sucrose and HF diet in the offspring rats (Vucetic et al, 2010) or increased anxiety-like behaviors in female offspring in primates (Sullivan et al, 2010). This was associated with a global and gene-specific promotor DNA hypomethylation and long-term alterations in gene expression (dopamine and opioids), or with epigenetic alterations of the serotonin signaling pathway, respectively. In humans, a recent epidemiological study (House et al, 2018) provided evidence for positive effect of a mediterranean maternal diet on behavioural outcomes in offsprings (decrease child depression, increase social relatedness). In parallel, the authors demonstrated associations of such a diet with changes in methylation in imprinted control regions of several genes in a sex-dependent manner in blood cord. In sheep, periconceptional undernutrition showed epigenetic effects in fetal hypothalamic POMC and GR genes, both involved in the regulation of energy balance and food intake. The consequences on offspring feeding behavior are suggested but not demonstrated (Begum et al, 2012). The above described studies indicate that breeding practice may have so far underestimated effects of behavior and welfare in livestock, and that appropriate care and nutritional management of the dams may be a positive lever to improve offspring behavior and welfare. More work in epigenetics field is definitely needed.

## **Conclusion**

In conclusion, it is now time to take into consideration the importance of epigenetic processes on the establishment of offspring phenotype, at the transgenerational and inter-generational levels. The better understanding of underlying mechanisms is essential. Nevertheless, effects of modifications of nutrition planes in livestock aiming at reducing greenhouse gas emission or at developing new nutriment need to be evaluated at the transgenerational level before they are implemented. This awareness may contribute to increase the number of epigenetic studies. In system biology approaches, clarify epigenetic part into phenotype construction may participate to selection of animals with a great plasticity in their response and adaptability to all modification of

environmental factors such as nutrition. At academic level, collectively, genome wide epigenetic information may participate to produce comprehensive maps of functional elements in genome and contribute to fill the genotype-phenotype gap as proposed FAANG consortium (FAANG Consortium 2015).

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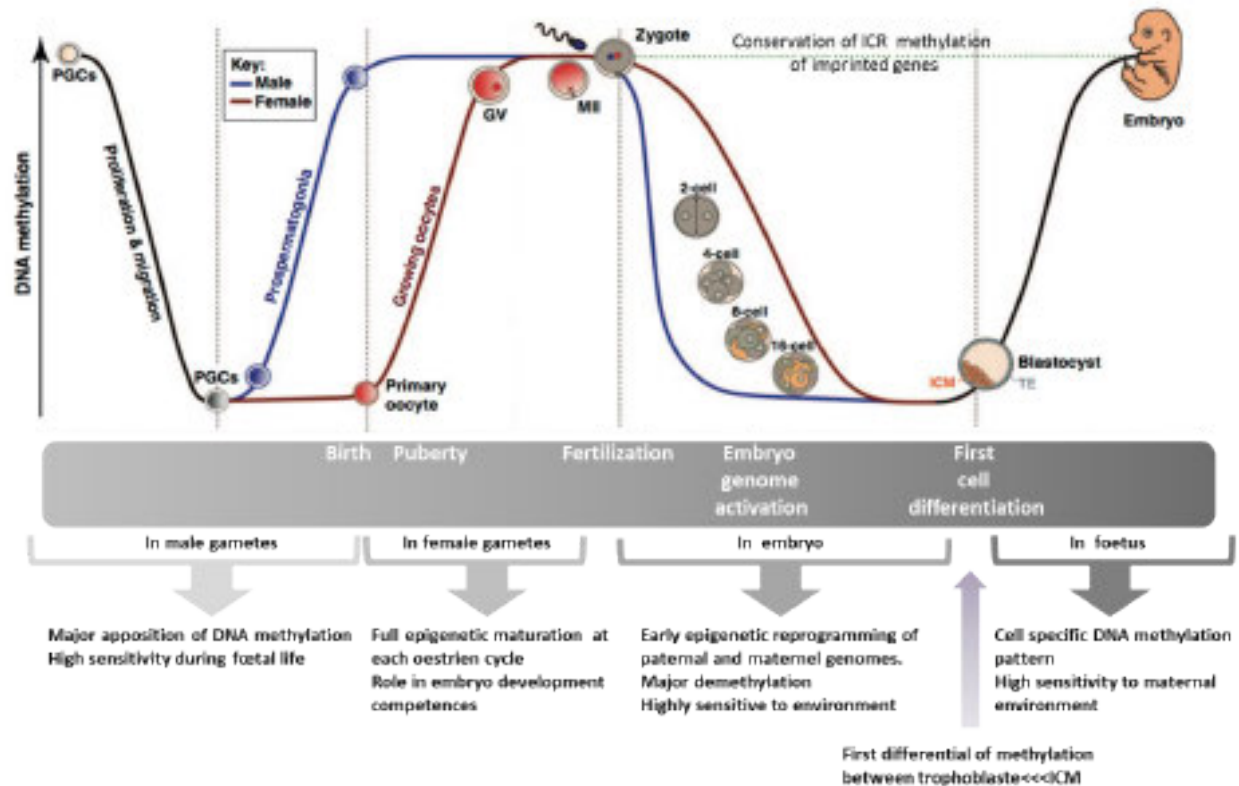
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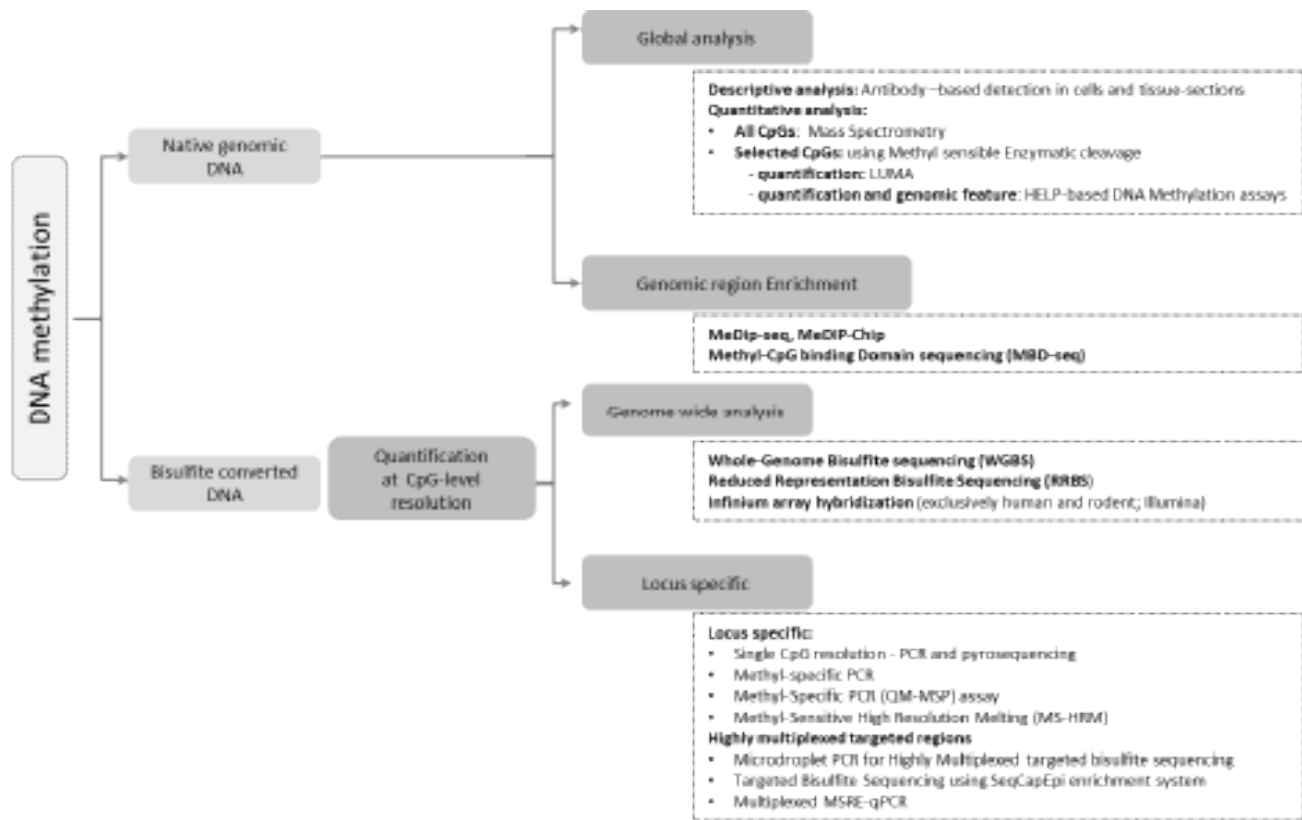
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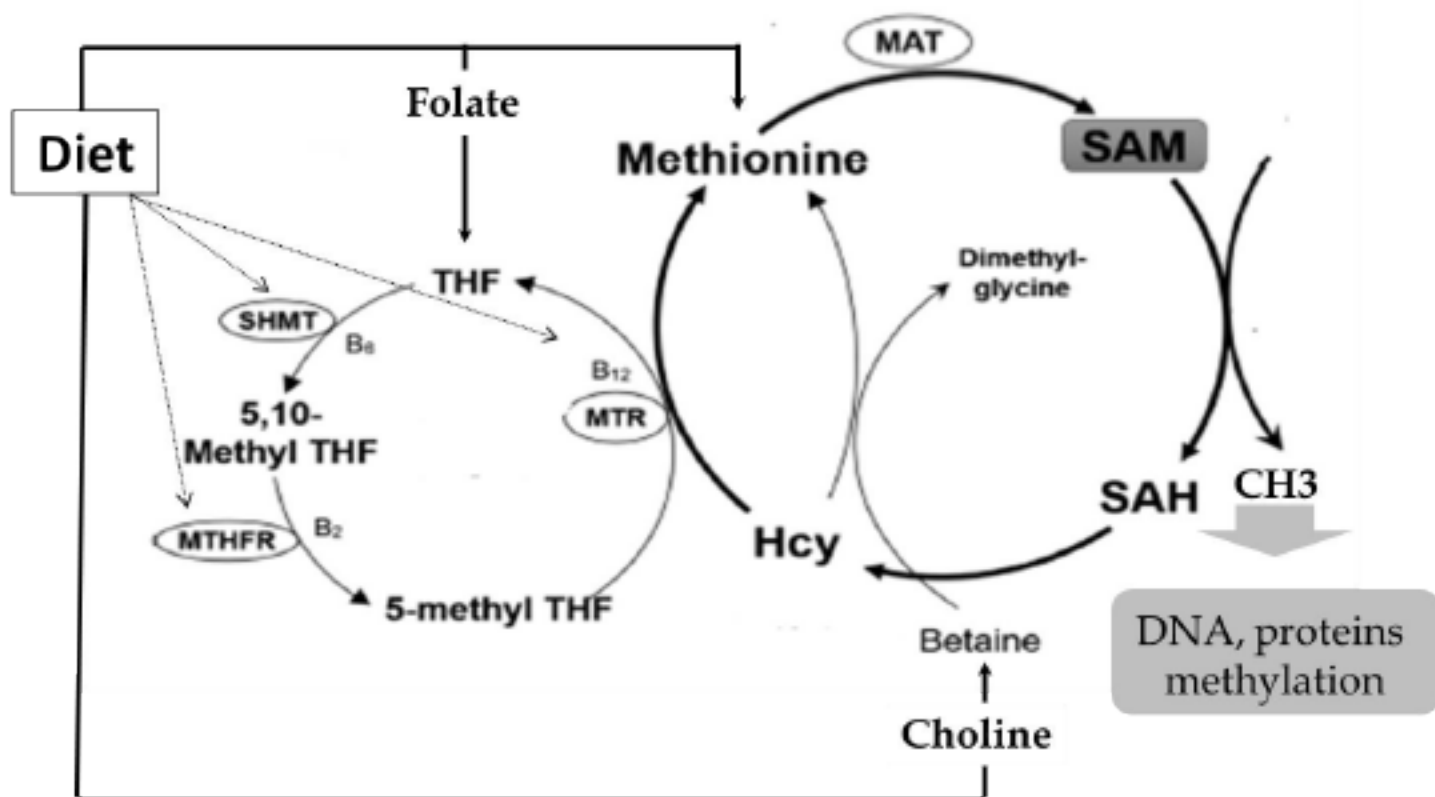
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**Figure 1.** Schematic representation of DNA methylation dynamics during development. The two major waves of DNA methylation loss occur. Firstly, in Primordial Germ Cells (PGCs) during the PGCs migration just before the gonadal differentiation. During male gametogenesis, the major DNA methylation is acquired at birth in spermatogonia and spermatocyte stages. Nevertheless, the full epigenetic maturity of sperm needs several histones PTM, histone-protamine replacement and nuclear compaction and occurs at each spermatogenesis cycle after puberty. In female, the primary oocyte displays a low epigenetic imprint. The full epigenetic maturity is acquired during the follicle maturation. The second wave occurs just after the fertilization: active and passive demethylation take place in paternal and maternal pronuclei, respectively. This reprogramming allows the establishment of totipotency in embryonic cells and the embryo genome activation. At blastocyst stage, the first lineage specific difference of methylation is observed: hypomethylation of trophoblast in comparison with inner cell mass (ICM). During gestation, the cell lineage concomitants with organogenesis are related with establishment of specific DNA methylation patterns. (adapted from Smallwood and Kelsey, 2012 and Chavatte-Palmer et al., 2016).



**Figure 2.** Schematic representation of large panel of most used methodologies to analyze DNA methylation. This representation is not exhaustive.



**Figure 3.** Schematic representation of “One carbon metabolism” pathways. Different components, directly involved in “one carbon metabolism” are originated from diet: Folate, Choline, Betaine, Methionine, vitamins B2, B6 and B12. SAM, S adenosyl methionine is the universal donor of CH<sub>3</sub> group to DNA (DNA methylation) as well as to proteins (at lysine and arginine residues of histone H3 and H4).

SAM = S-adenosyl methionine; MAT = methionine adenosyl transferase; SAH = S-adenosylhomocysteine; Hcy = homocysteine  
 THF= tetrahydrofolate; 5, 10-MTHF: 5, 10-methylenetetrahydrofolate; 5, methyl THF = 5-methyl-tetrahydrofolate SHMT = serine hydroxymethyltransferase; MTR = methyltetrahydrofolate-homocysteine methyltransferase;  
 MTHFR = methyltetrahydrofolate reductase.

**Table 1.** Nutritional elements with reported epigenetic effects, updated from Delage and Dashwood (2008), Canani et al., (2011), Yang et al., (2016), Chavatte-Palmer et al., (2018) and Huang et al., (2019).

General dietary changes	Sources	Effect on epigenetic machinery
<ul style="list-style-type: none"> <li>Proteins</li> <li>Lipids</li> <li>Sugar</li> </ul>	<ul style="list-style-type: none"> <li>Low protein diet</li> <li>High protein diet</li> </ul>	
“one carbon metabolism” components	Sources	Effect on epigenetic machinery
<ul style="list-style-type: none"> <li>Methionine</li> <li>Folic Acid (Vitamin B9)</li> <li>Vitamin B12</li> <li>Vitamin B6</li> <li>Choline</li> </ul>	<ul style="list-style-type: none"> <li>Nuts, Beans, Soy</li> <li>Beans, lentils, asparagus, spinach, broccoli, avocado, mangoes, lettuce, sweet corn, and oranges, and whole wheat bread</li> <li>Yeast</li> <li>Sweet potatoes, bananas, potatoes, avocados, and pistachios</li> <li>Cauliflowe , Mushrooms, Beet Greens</li> </ul>	<ul style="list-style-type: none"> <li>DNA methylation</li> </ul>
“one carbon metabolism” components	Sources	Effect on epigenetic machinery
Short chain fatty acids, butyrate	Digestion of crude fibre	Histone modificatio
<b>Phenolic compounds</b>	Soybean, Spices	<input type="checkbox"/> Regulating activity of HDACs, HATs, HMTs, HDMs and DNMTs
Apigenin	Parsley, celery, chamomile tea, oranges, thyme, onions	<input type="checkbox"/> Decrease of HDAC content, increase of H3 and H4 acetylation
<input type="checkbox"/> Curcumin	Curcuma longa (turmeric roots)	<input type="checkbox"/> Demethylation & miRNA expression modific - tion
<input type="checkbox"/> Epigallocatechin-3 Gallate (EGCG)	Green teal polyphenol	<input type="checkbox"/> miRNA expression and histone modification
<input type="checkbox"/> Genistein	Lupin, fava beans, soybeans, kudzu, psoralea	<input type="checkbox"/> Inhibitor of class I HDAC & HAT; inhibitor DNMTs
<input type="checkbox"/> Quercetin	Apple, tea, onion, nuts, berries, broccoli,	<input type="checkbox"/> DNA methylation
<input type="checkbox"/> Resveratrol	Blueberries, Cranberries, Red grapes, Eucalyptus, Spruce	<input type="checkbox"/> miRNA expression and histone modification
		<input type="checkbox"/> Activation of nicotinamide adenine dinucleotide (NAD <sup>+</sup> ) dependent deacetylase SIRT1;
		<input type="checkbox"/> Activation of sirtuins (class III HDAC)
<b>Organosulfur compounds</b>		
<input type="checkbox"/> Diallyl disulfide (DADS)	Garlic	<input type="checkbox"/> HDAC inhibitor, induction of histone hyper-acetylation
<input type="checkbox"/> Methylsulfinyl-hexyl isothiocyanat	Horseradish	<input type="checkbox"/> Histone modificatio
<input type="checkbox"/> Phenethyl isothiocyanate (PEITC)	Cruciferous vegetables	<input type="checkbox"/> HDAC inhibitor & demethylation
<input type="checkbox"/> Sulforaphane (SFN)	Cruciferous vegetables, Brocoli	<input type="checkbox"/> HDAC inhibitor & DNMT-inhibiting effects & miRNAs
<b>Other phytochemicals and derivatives</b>		
<input type="checkbox"/> Anacardic acid	Cashew nuts (Anacardium occidentale)	<input type="checkbox"/> Structurally related to salicylic acid, Histone modificatio
<input type="checkbox"/> Butein	Rhus verniciflua (stems)	<input type="checkbox"/> Histone modificatio
<input type="checkbox"/> Dihydrocoumarin	Melilotus officinalis (sweet clover)	<input type="checkbox"/> Histone modificatio
<input type="checkbox"/> Fisetin	Rhus toxicodendron (leaves)	<input type="checkbox"/> Histone modificatio
<input type="checkbox"/> Garcinol	Garcina indica (fruit)	<input type="checkbox"/> Histone modificatio
<input type="checkbox"/> Isoliquiritigenin	Glycyrrhiza glabra (licorice)	<input type="checkbox"/> Histone modificatio
<input type="checkbox"/> Luteolin	Sweet red pepper, celery, parsley	<input type="checkbox"/> Histone modificatio
<input type="checkbox"/> Piceatannol	Blueberries	<input type="checkbox"/> Histone modificatio
<input type="checkbox"/> Theophylline	Black and green tea	<input type="checkbox"/> Histone modificatio
<input type="checkbox"/> Valproic acid	Fermentation of dietary fiber in the colo	<input type="checkbox"/> H3 & H4 hyperacetylation, inhibition of HDACs activity
<b>Minerals</b>		
<input type="checkbox"/> Copper	Ubiquitous	<input type="checkbox"/> Histone modificatio
<input type="checkbox"/> Nickel	Ubiquitous	<input type="checkbox"/> Histone modificatio
<input type="checkbox"/> Zinc	Ubiquitous	<input type="checkbox"/> Histone modificatio
Selenium	Brown Rice, Sunflower seeds, beans, Oatmeal, spinach, Lentils, brazil nuts	<input type="checkbox"/> Inhibition of DNMT1 <input type="checkbox"/> Demethylation of specific promoters <input type="checkbox"/> Reduction of HDACs activity <input type="checkbox"/> Increase of histone acetylation

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# **The Impact of Early Nutritional Insults and Long-Term Consequences on Health**

## **Effets des carences nutritionnelles en bas âge et conséquences durables pour la santé**

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### **Abstract**

Early nutrition can have a profound impact on developmental outcomes and long-term health. The developmental origins of health and disease hypothesis has demonstrated that almost any early nutritional insult or deviation from the dietary 'norm' can potentially program metabolism permanently. This early programming is most likely mediated by epigenetics, which can be permanently altered within a critical window of development early in life, depending on the tissue and organism. The focus on epigenetics has been on methylation of DNA which relies on methyl (ie one-carbon) metabolism pathways. Methyl metabolism centers on methionine, which is used equally for protein synthesis and transmethylation reaction (which uses methionine-derived SAM as the primary methyl donor). However, transmethylation pathways compete for methyl groups and the bulk of dietary methionine is used for synthesis of creatine and phosphatidylcholine (PC), with only a minor component used for DNA methylation. The partitioning of methyl groups among these reactions can be dramatically altered by varying dietary methyl supply (ie methionine, betaine, choline, folate) and demand (creatine, PC). For example, feeding creatine can shift methylation to PC synthesis and spare methionine for protein synthesis, while feeding methyl-deficient diets can limit transmethylation pathways and alter DNA methylation and gene expression. If early diets vary in methyl nutrients during the critical window of development, then long-term programming of metabolism can occur which can permanently impact adult health. We need to understand the interaction of methyl-related nutrients in early-life diets to optimize nutrient composition and avoid altered epigenetic programming.

### **Résumé**

La nutrition en bas âge peut avoir un profond impact sur les résultats développementaux et sur la santé à long terme. L'hypothèse sur les origines développementales de la santé et des maladies a démontré que presque toutes les agressions nutritionnelles ou les déviations de la « norme » alimentaire en bas âge peuvent potentiellement programmer le métabolisme de façon permanente. Cette programmation en bas âge est le plus vraisemblablement médiée par l'épigénétique, qui peut être altérée de manière permanente à l'intérieur d'une fenêtre critique de développement au début de la vie, selon le tissu et l'organisme. L'accent sur l'épigénétique a été la méthylation de l'ADN, qui dépend des voies métaboliques du méthyle (c.-à-d. one-carbon). Le métabolisme méthylique

est centré sur la L-méthionine, qui est utilisée tant pour la synthèse des protéines que pour la réaction transméthylique (qui utilise SAM dérivé de la L-méthionine comme donneur primaire de méthyle). Cependant, les voies de la transméthylation se disputent les groupes de méthyle et la plus grande partie de la L-méthionine alimentaire sert à synthétiser la créatine et la phosphatidylcholine (PC), avec seulement une composante mineure utilisée pour la méthylation de l'ADN. La séparation des groupes de méthyle parmi ces réactions peut être altérée considérablement en variant l'apport alimentaire de méthyle (c.-à-d. L-méthionine, bétaine, choline, folate) et la demande (créatine, PC). Par exemple, la créatine dans les aliments peut changer la méthylation en synthèse de la PC et garder la L-méthionine pour la synthèse des protéines, alors que les régimes alimentaires pauvres en méthyle peuvent limiter les voies de transméthylation et altérer la méthylation de l'ADN et l'expression génétique. Si les régimes en bas âge varient en nutriments méthyliques durant la phase critique de développement, alors la programmation du métabolisme à long terme peut se produire, ce qui peut influencer de façon permanente la santé à l'âge adulte. Nous devons comprendre l'interaction des nutriments liés au méthyle dans les régimes en début de vie pour optimiser la composition des nutriments et pour éviter d'altérer la programmation épigénétique.

## **Introduction**

Early programming refers to the long-term consequences of a nutritional or environmental insult early in life. Epidemiological studies have been able to link early nutrition insults with several adult chronic diseases such as obesity, hypertension, cardiovascular disease, Type 2 diabetes and cognitive outcomes (McMillen and Robinson, 2005). These early nutritional insults can be quite varied and it would appear that any major alteration to normal nutrition in early development has the potential to permanently program metabolism. The concept that early nutrition can 'program' metabolism and permanently increase the risk for later disease has led to a profound paradigm shift in how we think about feeding practices during development. Indeed, this plasticity in metabolic responses to the environment is key to the predictive adaptive response and is an essential tool for successful adaptation. However, inevitably, these adaptive responses come at a cost. If we can understand the mechanisms by which early nutritional programming occurs, then early diets can be designed to offset the risk for diseases later in life.

## **Developmental Origins of Health and Disease**

The 'developmental origins of health and disease' hypothesis describes the long-term consequences of a nutritional or environmental insult during early development. The permanent effects of an insult during a critical period in utero or during postnatal development has been recognized for many years in many species (Metcalf and Monaghan, 2001). However, evidence from human epidemiological studies are more recent. Initial studies linked small birth weight and rapid postnatal compensatory growth to various chronic adult diseases including obesity, hypertension, coronary heart disease, Type 2 diabetes and dyslipidemia (Barker, 2007). In spite of the abundant recent data supporting these associations, this developmental programming does not account for the global epidemic in adult chronic diseases. Rather, it is the risk or susceptibility for developing diseases in response to nutritional stressors that is being programmed. In other words, it is our tolerance to these stressors which is determined early in life in a manner that allows the organism to adapt its metabolism to a predicted nutrition environment.

The early epidemiological evidence was based on retrospective correlational studies by David Barker and colleagues that associated infant mortality and low birth weight with incidence of chronic diseases in various populations (Barker, 2007). Similar associations were found in the notorious Dutch Famine studies, which followed individuals who were born to mothers exposed to the six-month Dutch winter hunger during World War II. These studies were able to refine the associations of specific diseases with severe food restriction in specific trimesters of pregnancy (Painter et al., 2005). These original associations led to what was then termed the “Barker” or fetal programming hypothesis, which postulated that intrauterine stresses to a fetus programmed its metabolism permanently for a postnatal environment that was predicted to be just as stressful (Godfrey et al., 2007). If there was a mismatch in the predicted environment from the actual environment, then the adaptive response was inappropriate and risk for disease increased. For example, if a fetus experiences poor nutrition from a malnourished mother, then its metabolism is programmed to be more efficient with nutrients since it predicts a poor nutritional environment after birth; this adaptation has obvious evolutionary advantages. If the infant predicts its postnatal environment wrongly and is exposed to nutritionally rich environment, then this efficient metabolism becomes a disadvantage. These concepts were further developed with studies associating incidence of chronic diseases with postnatal growth rates. In many populations, the incidence of disease was particularly significant in people who were born smaller, but who also grew faster in childhood (Eriksson, 2019). This compensatory growth after intrauterine growth retardation is known to affect long term outcomes in many species (Metcalf and Monaghan, 2001), and has been confirmed in various human populations. Indeed, the combination of smaller birthweight and rapid postnatal growth rate is more associated with disease outcomes than either characteristic alone.

Since those original epidemiological studies, many animal models have been developed to explore mechanisms of programming (McMullen and Mostyn, 2007). These models vary widely in the type of early nutritional stress and the disease outcome measured. The effects vary depending on the tissue studied, the metabolic outcome measured and the timing of stress. The many models developed come to the general conclusion that almost any early nutritional insult or deviation from the dietary ‘norm’ can potentially program metabolism permanently (Field, 2009). The question is what mechanism can account for this wide plasticity of outcomes from a seemingly endless list of nutrition perturbations?

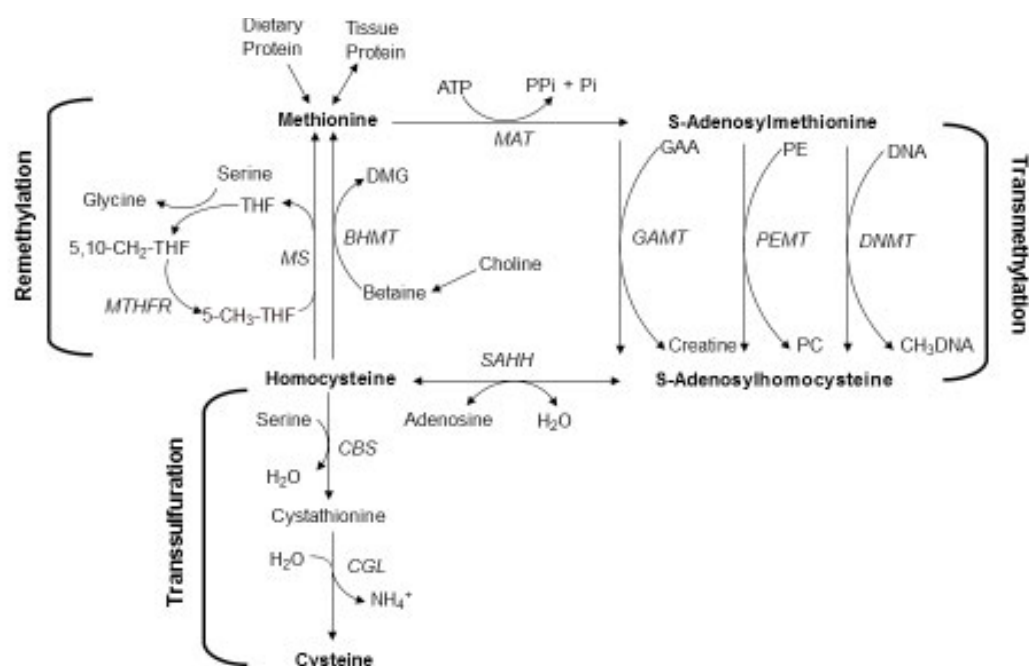
## **Mechanism of Programming**

The mechanism of programming of metabolic pathways is most likely mediated by epigenetics. Epigenetic modification of gene expression has many key characteristics that match the characteristics of the developmental origins of health and disease hypothesis. For example, epigenetics can modify gene expression in gradations and is modifiable during developmental periods in all tissues. Moreover, epigenetic patterning can be modified by many environmental cues, including nutritional perturbations, but can only be modified during specific ‘windows’ of development. And finally, epigenetic patterns are conserved during cell division which allows changes to these patterns to be established early and become permanent after these ‘windows’ are closed. There are several types of epigenetic modifications, but the most relevant mechanism with respect to the current discussion involves methylation of DNA, which occurs via DNA methyltransferases (DNMTs) utilizing S-adenosyl methionine (SAM) as the methyl donor.

DNA methylation occurs in many parts of the genome with the bulk of methylation found in association with repetitive sequences to silence genes. But coding sequences can also be methylated and there is strong evidence to suggest that this is an important form of gene control. Indeed, recent work has suggested that promoter-specific epigenetic regulation may be a key process in gene regulation for many proteins (Vickers, 2014). Tissue-specific, heritable methylation patterns are established at different times for different sets of genes throughout early development up to and including the neonatal phase. However, the most exciting findings connecting epigenetics to programming are that methylation patterns can be altered by dietary methyl supply in utero (Waterland and Jirtle, 2003) and postnatally (Waterland et al., 2006), and that epigenetic changes due to early programming are reversible with dietary methyl supplementation (Burdge et al., 2009). Because DNMTs use SAM, the primary methyl donor in the body, other transmethylation enzymes, many of which consume vastly more SAM, will compete for the same methyl groups. How is this partitioning of methyl groups regulated and which transmethylation pathway has priority when substrates are deficient

## **Methyl Metabolism**

Methyl metabolism centers on the essential amino acid methionine, which is used equally for protein synthesis and transmethylation reactions (via SAM as the primary methyl donor). In **Figure 1**, the pathways for methionine metabolism are presented and can be summarized by transmethylation (TM: methionine to homocysteine), transsulfuration (TS: homocysteine to cysteine) and remethylation (RM: homocysteine to methionine). Briefly, methionine is adenylated to SAM which is the methyl donor in over 50 different reactions. The end-products are the methylated compound and S-adenosylhomocysteine, which is converted to homocysteine. Homocysteine is either irreversibly catabolized to cysteine (and taurine) via the TS pathway or remethylated to methionine. RM of homocysteine to methionine occurs either via Vitamin B<sub>12</sub>-dependent methionine synthase (MS) and folate, or via an equally important route, BHMT, which uses betaine, a product of choline oxidation, as the methyl donor. There is evidence that these RM pathways can compensate for each other to some extent; indeed, we have shown that either folate or betaine can equally increase RM and methionine availability in the piglet (McBreairty et al., 2016; Robinson et al., 2018). The regulation of these methionine pathways is complex (Finkelstein, 2000). When cysteine is unavailable, TM and TS are increased to oxidize methionine and synthesize cysteine. Similarly, when methionine is in excess, TM and TS are upregulated to oxidize methionine which is toxic at relatively low concentrations. When methionine is scarce, then TS is downregulated and RM is upregulated to regenerate methionine. However, the nutritional consequences of these changes are less clear, including how methylated products spare SAM and methionine for other uses.



**Figure 1. Methionine metabolism pathways. (McBreairty and Bertolo, 2016)**

BHMT, Betaine-homocysteine methyltransferase; CBS, Cystathionine  $\beta$ -synthase; CGL, Cystathionine  $\gamma$ -lyase; DMG, Dimethylglycine; DNMT, DNA methyltransferase; GAA, Guanidinoacetate; GAMT, GAA methyltransferase; MAT, Methionine adenosyltransferase; MS, Methionine synthase; MTHFR, Methylenetetrahydrofolate reductase; PE, Phosphatidylethanolamine; PC, Phosphatidylcholine; PEMT, PE *N*-methyltransferase; SAHH, *S*-adenosylhomocysteine hydrolase; THF, tetrahydrofolate

## Dietary Methyl Supply and Demand

The non-protein requirement for methionine can be viewed as the TM requirement of the body; as long as cysteine intake is adequate, then the requirement for methyl groups will drive the non-protein requirement for methionine (Bertolo and McBreairty, 2013). Of the more than 50 methylation reactions, the most important enzymes are: 1) phosphatidylethanolamine methyltransferase (PEMT) which synthesizes endogenous phosphatidylcholine (PC); 2) guanidinoacetate (GAA) methyltransferase (GAMT) which synthesizes creatine; and 3) DNMT which methylates DNA. GAMT and PEMT have a substantial methyl requirement, especially in growing animals that are rapidly expanding their tissue pools. Put in perspective, our data show that creatine synthesis *alone* obliges a TM rate of 640  $\mu\text{mol/kg/d}$  in the piglet (Brosnan et al., 2009) compared to the total TM flux (*for all TM reactions*) of 170  $\mu\text{mol/kg/d}$  in adult humans (Hoffer, 2002). We have recently calculated partitioning of dietary methionine and found that in suckling piglets, of the dietary methionine metabolized by the liver, only 10-30% ends up in protein with the vast majority of the remainder found in creatine and PC (Tables 1 and 2). And of course, diet has a significant impact on this partitioning; for example, a diet low in creatine (eg, plant foods) can result in a dramatic re-partitioning of methyl groups. In fact, our recent data demonstrate that PC synthesis is most sensitive to methyl supply. When GAA is infused (Table 1) or fed (McBreairty et al., 2015) to induce creatine synthesis, PC synthesis via PEMT is dramatically reduced due to limited supply of methyl

groups (McBreairty et al., 2013 and 2015; Table 1), while hepatic protein synthesis is conserved (Tables 1 and 2). And although the methylation of DNA via DNMT accounted for less than 1% of dietary methionine, its developmental importance and sensitivity to dietary manipulation makes this methylation pathway perhaps the most important of all, with respect to the long term health of the organism.

**Table 1.** Percent of  $^3\text{H}$ -methyl-labelled product remaining in the liver 60 min after infusion with of L-[methyl- $^3\text{H}$ ]methionine in piglets under control or infused with guanidinoacetate (high methyl demand) (Adapted from McBreairty et al., 2013).

		High Methyl Demand
(%)	Control	
Protein	32	26
Creatine	18	66
PC	49	8
DNA	0.10	0.09

**Table 2.**  $^3\text{H}$ -Methyl products remaining in the liver after 6 h constant infusion with L-[methyl- $^3\text{H}$ ]methionine (dpm x 1000/g liver) in piglets fed diets low (80% of requirement) or high (200% of requirement) in methionine and arginine for 7 days (unpublished data).

	Low Met/Arg	High Met/Arg
(dpm/g)		
Protein	66	67
Creatine	74	202*
PC	137	343*
DNA	8	11*

Diet composition will affect the relative requirements of these pathways. Moreover, during growth and development, all of these methyl-consuming pathways are highly active not only to maintain basic functions, but also to expand the pools of their respective products to meet the demands for growth. For example, a creatine-free diet (eg, plant foods) will require the organism to synthesize its entire requirement for creatine, thereby consuming significant amounts of the essential amino acids methionine and arginine (Brosnan et al., 2009). Moreover, foods relatively poor in methionine (eg, legumes) will require enhanced remethylation and the nutrients associated with those pathways (folate, Vitamin B12, choline, betaine, serine). Ultimately, these pathways depend on the methyl supply, both from the diet and from de novo synthesis. If one or two of these interrelated nutrients are limiting, then the other(s) must compensate to maintain supply. Because of its importance

in protein synthesis, the dietary methionine requirement has always been carefully determined using protein synthesis outcomes, but not with respect to the abundance of dietary methyl donors. The requirement for methionine must accommodate not only its flux to protein and cysteine, but also its flux to transmethylation products, fully considering its remethylation back to methionine. The partitioning of methyl groups among these reactions can be dramatically altered by varying dietary methyl supply (ie, methionine, betaine, choline, folate) and demand (eg, creatine, PC). For example, feeding creatine can shift methylation to PC synthesis and spare methionine for protein synthesis, while feeding methyl-deficient diets can limit TM pathways (Robinson et al., 2016) and alter DNA methylation and gene expression. It is clear that there are many variables that can affect methionine requirements, which in turn can affect epigenetic programming of long term health and performance.

## Final Thoughts

If early diets vary in methyl nutrients during the critical windows of development, then long-term programming of metabolism can occur which can permanently impact adult health. The critical window is obviously during development, but evidence shows it varies considerably for different tissues and different functions. For example, brain development continues into adolescence and so susceptibility to permanent epigenetic modification is likely extended compared to renal function, which is established by late gestation. Although unclear, it is also likely that the sensitivity of each tissue to dietary perturbations is also very different.

In most animals, the nutritional requirements for the fetus during gestation is generally adequate, often at the expense of the mother's health. Similarly, during suckling, neonates are somewhat buffered by a consistent nutrient profile of breast milk, sometimes at the expense of maternal nutritional status. Although the fetus and suckling neonate are generally protected from major nutritional perturbations during early life, there are likely various nutrient-specific perturbations that could have unseen epigenetic effects. Moreover, the weaning and pre-sexual maturity phases of growth are particularly vulnerable periods of development since diets during these phases vary considerably, especially considering diets in growing animals are typically designed around growth outcomes, rather than general health and resistance to future challenges. The animal industry in particular needs to reconsider the non-growth requirements of certain nutrients (including non-required nutrients such as betaine and creatine) and avoid the mentality that 'if it's not toxic, more is safer'. Imbalance of these critical nutrients is as important as abundance and many unnoticeable changes during development can dramatically alter performance and health later in life.

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# Graduate Student Posters

## *Affiches des étudiant(e)s diplômés*



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## Does Supplemental Methionine and Protein Improve Performance and Digestibility During Late-Gestation in Beef Cows?

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### Abstract

Methionine (Met) is likely the first limiting amino acid in low-protein forages fed to beef cattle during late-gestation. Therefore, the objective of this study was to determine if supplemental protein and Met improves cow performance and total tract digestibility during late-gestation. This study used 147 late-gestation Angus crossbred cows and heifers in a 3 x 2 factorial arrangement for dietary treatments. The cattle were randomly assigned to one of six diets formulated to 90, 100 or 110% of metabolizable protein (MP) requirements (NRC, 2016), with (without) 9 g/d of rumen-protected Met fed for approximately 8 wks before calving. Cows fed at 90% MP requirements lost body weight, while cows fed at 100% and 110% MP requirements maintained and/or gained body weight over the trial period ( $P=0.02$ ). Cows fed at 90% MP requirements had reduced apparent tract digestibility for crude protein ( $P<0.01$ ). However, supplemental Met did not improve body weight gain or apparent tract digestibility values ( $P>0.16$ ). Calf birth weights were not impacted by dietary treatment ( $P>0.31$ ). Met supplementation reduced serum concentrations of 6 amino acids including isoleucine, leucine, lysine, serine, threonine and valine ( $P<0.03$ ). Feeding cows above their MP requirements may improve late-gestation performance. Supplemental Met may increase amino acid utilization but did not improve cow performance parameters measured in this experiment.

**Keywords:** beef cows, gestation, metabolizable protein, methionine

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## Effects of Management Regimen and RFI Classification on Steer Feeding Behaviour and Growth Performance

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### Abstract

One-hundred and eight, crossbred steers (non-implemented) were used to evaluate the effects of backgrounding and residual feed intake (RFI) classification on feeding behaviour and growth performance to ultimately lower production costs. Steers were fed a growing ration (alfalfa/corn silage) for 90 days then allocated to one of two management regimen (MR) based on growing phase RFI (high, medium, low), weight, and breed (British/Continental). MR 1 cattle were fed an 84.7% concentrate finishing ration. MR 2 cattle were backgrounded on pasture for ~112 days before being finished on the same concentrate ration as MR 1 cattle. RFI was calculated for each steer at the end of growing and finishing phases along with evaluating performance and behaviour traits based on MR and RFI classification. Finishing phase average daily gain and G:F for MR 1 were 1.80 kg/day and 0.15, respectively versus 1.92 kg/day ( $P<0.02$ ) and 0.15 respectively for MR 2 cattle. Feeding behaviour in the growing phase found low RFI steers visited the feeder less, spent less time at the feeder, and had a slower eating rate with less feed consumed per visit than high RFI steers ( $P<0.02$ ). In the finishing phase, growing phase RFI classification did not affect finishing phase feeding behaviour traits. However, based on finishing phase RFI classification, low RFI steers consumed less meals per day ( $P<0.001$ ) with a trend for a slower eating rate ( $P<0.10$ ) than high RFI steers. Most feeding behavior traits were affected by backgrounding ( $P<0.02$ ). In addition, MR by RFI interactions ( $P<0.08$ ) were present for several feeding behavior traits. Evaluation of feeding behavior data as affected by MR may help us explain how different MR can affect efficiency of feed conversion

**Key words:** beef cattle, backgrounding, feeding behaviour, residual feed intake, performance

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# Assessing the Relationship Between Cow-Calf Management Systems and Metabolic Programming on the Performance of Cow-Calf Pairs Prior to Weaning

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## Abstract

Quantity or quality of nutrients consumed by beef cows during gestation may influence calf performance. Rapid fetal growth occurs as cows prepare for lactation, increasing protein and amino acid requirements. Management may alter cow-calf performance prior to weaning, as drylot systems provide more control of nutrition and health while pasture systems may reduce labour and feed costs. This study assessed the impact of metabolic programming during late gestation, and two lactational management systems on the performance of cow-calf pairs prior to weaning. Eight weeks prior to parturition, 140 Angus crossbred beef cows were managed in a drylot system and fed to meet 110%, 100%, or 90% of the metabolizable protein requirements for late-gestation, with (without) rumen bypass methionine (RBM). Post-partum, cow-calf pairs were managed in drylot (DL) or rotationally grazed on pasture (PAS). Cow body weight (BW) and body condition score (BCS) were recorded every 28 days until weaning. Calf BW was recorded at birth and regular intervals up to and including weaning. Changes in cow BW and BCS during lactation were not affected by gestational nutritional program (protein level, RBM), or management system during lactation (DL, PAS) ( $P>0.05$ ). Prenatal dam protein level and RBM supplementation did not impact calf BW prior to weaning ( $P>0.20$ ). Lactational management system significantly affected calf BW, as PAS calves were heavier than DL calves throughout the pre-weaning period ( $P=0.02$ ). In conclusion, metabolic programming did not affect cow performance during lactation and calf performance prior to weaning. However, rotationally grazing cow-calf pairs may improve pre-weaning gains in calves, resulting in potential economic benefits for producers

**Keywords:** *metabolic programming, protein requirements, rumen bypass methionine, rotational grazing, confinement system*

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## Effects of Protein and Supplemental Methionine in Late Gestation on Colostrum Quality and Passive Immunity Transfer in Beef Cattle

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### Abstract

The five weeks leading up to parturition represent the time frame in which bovine maternal antibodies are transferred into colostrum from the dam's blood. Therefore, limiting nutrients to the cow during late gestation may impact colostrum production and quality. Methionine is considered to be a prominent limiting amino acid for beef cattle, and its availability may impact colostrum protein production. The objective of this study was to evaluate the influence of metabolizable protein (MP) and supplemental rumen-protected methionine (RPM) during late gestation for beef cows on colostrum quality and passive immunity transfer. One hundred and fifty-one Angus crossbred cows and heifers were randomly assigned to one of six dietary treatments based on a 3 by 2 factorial arrangement. Diets included meeting 90%, 100% and 110% MP requirements according to NRC (2016) recommendations, offered with (without) RPM (9 vs. 0 g/d). Cattle were individually fed a partially mixed ration (60% haylage/40% straw for cows, and 70% haylage/30% straw for heifers), and top-dressed supplements daily, for approximately 56 d prior to calving. While MP level during gestation did not affect total protein or IgG concentrations in calf serum ( $P \geq 0.64$ ), providing supplemental RPM decreased total protein (5.79 vs.  $6.33 \pm 0.18$ ;  $P = 0.0022$ ) and IgG concentrations in calf serum ( $34.37$  vs.  $42.99 \pm 3.37$ ;  $P = 0.01$ ). Level of MP, RPM, or their interactions did not influence concentrations of IgG, fat, protein, BHB or SCC in colostrum ( $P \geq 0.12$ ). Supplementation of RPM decreased concentrations of MUN ( $45.92$  vs.  $54.51 \pm 4.13$ ;  $P = 0.04$ ). These results suggest providing supplemental methionine for beef cows during gestation may alter colostrum quality and decrease protein and antibody levels in developing offspring.

**Keywords:** protein, methionine, colostrum, serum, beef calves

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## Alfalfa Establishment: Evaluating the Effects of Underseeding Sudangrass and Ryegrass on Forage Yields and Chemical Composition

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### Abstract

Poor forage yields and quality in the first year of establishing alfalfa (*Medicago sativa* L.) are major challenges in dairy cow nutrition. This study evaluated the effects of underseeding alfalfa with different annual companion crops on forage yield, botanical composition, chemical composition, alfalfa establishment and persistence. Treatments included alfalfa seeded in solo (control) or with a companion forage (Sudangrass, ryegrass or oat). Experimental plots (4 per treatment) were seeded twice (in May and June) and in two different environments. All plots were harvested (3 and 2 times in May and June, respectively) at the budding stage of alfalfa for total yield determination (DM basis) and manually separated and weighed by forage type. Forage subsamples were analyzed for NDF, ADF and CP. Alfalfa establishment and winter survival were determined by measuring alfalfa stem density in the fall of the seeding year and after one winter (spring) in the following year. Data were analyzed using the GLM procedure of SAS for each environment. In both environments, total forage yields were highest ( $P < 0.05$ ) with Sudangrass (3.6T/ha) and lowest ( $P < 0.05$ ) with control (2.4T/ha) or ryegrass (2.1T/ha). Alfalfa yield was markedly reduced ( $P < 0.05$ ) with oat when compared to other companion crops. For June established plots, yield of companion crop was higher with Sudangrass (average 1.6T/ha) and oat (1.2T/ha) than ryegrass (0.2T/ha). Sudangrass and oat produced forages with lower ( $P < 0.001$ ) CP but higher ( $P < 0.001$ ) NDF and ADF levels than control and ryegrass. Calculated relative feed values of forages were highest ( $P < 0.001$ ) with control and ryegrass treatments. Sudangrass yielded more CP (1.4x) and NDF (1.9x) than control per hectare of cultivated land. Companion crops had no detrimental effects on alfalfa establishment and persistence. In conclusion, underseeding alfalfa with Sudangrass may improve both forage and NDF yields for better cow's nutrition.

**Keywords:** alfalfa, companion crops, dairy cows

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## Establishing Perennial Forages with Annual Sudangrass or Sorghum-Sudangrass Hybrids Improved Forage Yields and in Vitro Total-tract NDF Digestibility

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### Abstract

In Canada, alfalfa fields are usually established together with different perennial gramineae. However, in the first year, poor forage yields and quality greatly affect cow's nutrition. This study evaluated the effects of underseeding perennial forages (alfalfa, clover and tall fescue; control) with different annual companion forages on forage yields, chemical composition and total-tract NDF digestibility (TTNDFD) using Daisy<sup>II</sup> incubator. Treatments were the control seeded alone or with a companion forage [Sudangrass (SG), Sudangrass brown midrib [BMR] (BSG), Sorghum-Sudangrass BMR (BSSG) or oat]. Experimental plots (7 per treatment) were harvested at d 60 (1st cut) and d 90 (2nd cut) at bud stage of alfalfa. Forage indigestible NDF (iNDF) was calculated by in vitro incubation at 240 h and potentially degradable NDF (pdNDF) was calculated by subtracting iNDF from total NDF. Data were analyzed using the MIXED procedure of SAS with fixed effects of treatment, cut and treatment x cut interaction. Total forage yields (cuts 1 and 2; DM basis) were higher ( $P < 0.001$ ) with SG (6.56T/ha), BSG (5.50T/ha) and BSSG (5.37T/ha) than control (2.82T/ha). Oat produced higher forage yield in the first cut only. In presence of companion crops, yields of individual perennial forages and weeds were reduced ( $P < 0.001$ ). Companion crop yield was lowest ( $P < 0.001$ ) with oat (3.49T/ha), intermediate with BSG (4.72T/ha) and highest with SG (5.86T/ha) or BSSG (5.89T/ha). Companion forages reduced ( $P < 0.001$ ) CP and ADL but increased ( $P < 0.001$ ) NDF and ADF levels of harvested forages. In vitro TTNDFD followed the order ( $P < 0.001$ ): BSG and BSSG (average 59.2%) > SG (55.7%) > control (49.9%) > oat (46.0%). Evidently, iNDF was lower ( $P < 0.001$ ) with SG, BSG and BSSG than control and oat. In conclusion, seeding perennial forages with SG, BSG or BSSG may improve both forage yields and fiber digestibility for better cow's nutrition.

**Key words:** dairy cows, forage, fiber digestibility

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# Adding Yeast to the Diet of Late Finishing Beef Steers Improves Feed Conversion Without Compromising Gains or Carcass Quality

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## Abstract

Feed represents the number one cost for beef production and is an area in which producers strive to improve for increased profitability. Previous research indicates that in the life of a growing beef steer the highest period of ruminal acidosis risk occurs in the late finishing phase. Ruminal acidosis creates an economic burden for producers by increasing veterinary costs and production losses for cattle. Adding yeast to diets can stabilize rumen pH by reducing lactate accumulation to return the rumen to homeostasis. The objectives of this study are to investigate the impact of supplementing a high dose of yeast (60 billion *Saccharomyces cerevisiae* colony forming units) on performance, carcass traits, and indicators of rumen health in late finishing feedlot steers. To meet these objectives, 54 steers were fed a high-moisture corn-based finishing diet in pens equipped with Insentec feeders to record individual animal feed intake and behaviour. Yeast supplementation decreased dry matter intake by 31% ( $P < 0.001$ ) while maintaining similar average daily gains ( $P = 0.50$ ) to control steers, thus improving feed conversion ( $P < 0.001$ ) for steers fed the diet with added yeast. Feeding behaviour data indicated a significant decrease in visits to the feeder per day and meal size ( $P \leq 0.005$ ) for yeast fed steers. Additionally, there were no differences between groups for rumen health ( $P \geq 0.08$ ), immune response indicators ( $P \geq 0.91$ ), blood metabolites ( $P \geq 0.13$ ), or carcass characteristics ( $P \geq 0.15$ ). This study has demonstrated a high dose of yeast added to the late finishing diet results in significant improvements to feed conversion through a dramatic decrease in dry matter intake without impacting animal performance or carcass quality. Therefore, the addition of yeast may be a natural feed additive which has the potential to increase profitability for feedlot producers.

**Keywords:** yeast, growth performance, beef

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## Improvement in Skeletal Development of ISA-Brown Pullets by Maternal and Post-hatch Feeding of Omega-three Fatty Acids

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### Abstract

To try to cope with skeletal problems such as osteoporosis in laying hen industry, nutritional approaches are being used either at age of maturity or when there is high risk for osteoporosis. However, the embryonic period is critical for future metabolism and growth. Therefore, nutritional modification during pre-hatch period may improve a bird's health. Limited research has investigated maternal and early life feeding of  $\omega$ -3 fatty acids ( **$\omega$ -3FA**) on skeletal development in the progeny. Thus, this study aimed to evaluate the effects of maternal and post-hatch dietary  $\omega$ -3 FAs on skeletal development in pullets. ISA brown breeders at 26 weeks of age were fed by three maternal treatments including 1) Control (C), 2) supplementation with 1% of a dried micro-algae (*Aurantiochytrium limacinum*) fermentation product (A), as a source of docosahexaenoic acid, and 3) supplementation with 2.48% of LinPro, a dry extruded product consisting of full-fat flaxseed (L), as a source of alpha-linoleic acid. Test diets (A and L) had equal amounts of  $\omega$ -3 and ratio of  $\omega$ -6:  $\omega$ -3. Offspring of maternal C were divided into three post-hatch treatments: C (C-C), A (C-A) and L (C-L). Offspring of maternal A were divided into two post-hatch treatments: C (A-C) and A (A-A). Offspring of maternal L were divided into two post-hatch treatments: C (L-C) and L (L-L). Pullets were necropsied at 12 wk for tibia and femur sampling. Left tibia and femur epiphysis were separated from diaphysis. Diaphysis was cut longitudinally the medullary bone removed by scraping and the remainder designated as cortical. The dried weight (DW) and ash weight (AW) were measured in epiphysis, cortical and medullary while the right tibia breaking strength was measured by the Instron machine as the amount of applied Newton (N/m<sup>2</sup>) pressure at 2 mm/sec. There was no effect on total DW, AW and ash percentage of tibia and femur ( $P > 0.05$ ). Pullets from the L-C group had more AW (11.90 %,  $P = 0.047$ ) in tibia cortical compared to the C-C group. The L-C pullets had stronger tibia ( $P = 0.038$ ) compared to pullets from the C-C group and those from the A-A group. There was a positive correlation between cortical AW and breaking strength ( $r = 0.308$ ,  $P = 0.046$ ). These findings demonstrated the effectiveness of maternal feeding of  $\omega$ -3 FAs as more efficient feeding strategy over rearing feeding program in support of skeletal strength in young pullets. The inclusion  $\omega$ -3 source into either maternal and post-hatch diets did not improved skeletal strength.

**Keywords:** maternal feeding, pullets, bone development,  $\omega$ -3 FA, fatty acids,

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## Physiochemical Characterization and Digestible Energy of Ethanol Co-products Streams for Use in Swine Diets

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### Abstract

Dried distillers' grains with solubles (**DDGS**) are co-products produced by the (corn) ethanol industry, which contain relatively high fibre and low energy availability for monogastric animals. Ethanol plants are implementing pre-fermentation fibre fractionation technologies to improve fermentation efficiency and potentially energy availability, producing a novel, high protein DDGS (**HiPro**). To determine the impact of pre-fermentation fibre fractionation, the physiochemical properties of HiPro were evaluated and compared to DDGS and the parent crop (corn). The digestible energy contents of HiPro and DDGS with and without fibre-degrading enzymes (i.e. multi-carbohydrase) were also determined for growing pigs (n=7). HiPro had half as much starch as DDGS (2.6 vs. 5.2%, respectively; dry matter basis); the starch concentration of corn was 78.0%. HiPro had 17% more protein than DDGS (36.6 vs 30.4%, respectively); the protein concentration of corn was 7.6%. The bulk density of HiPro was 6% less than that of DDGS (538 vs 568 g/L, respectively; as-fed) and 9% less than that of corn (607 g/L), suggesting that HiPro is less compact. The swelling capacity of HiPro and DDGS did not differ (3.6 L/kg vs. 3.5, respectively) but were both ~32% greater than that of corn (2.4 L/kg). The water binding capacity of HiPro was 12% greater than that of DDGS (3.3 vs 2.90 g/g, respectively) and 38% greater than corn (2.1 g/g), indicating the presence of more soluble versus insoluble fibre in HiPro. The digestible energy content of HiPro was greater than DDGS for growing pigs (3652 vs 3262 kcal/kg;  $P < 0.05$ ) and exogenous fibre-degrading enzymes were ineffective at improving digestible energy of either HiPro or DDGS. HiPro has different physiochemical properties than either DDGS or corn, which will likely influence HiPro use in swine diets; to optimize digestible energy for growing pigs, different exogenous feed enzymes are likely required for HiPro versus DDGS.

**Keywords:** swine, ethanol co-products, physiochemical properties, digestible energy

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## Digestive and Post-absorptive Utilization of Dietary Crude Protein Was not Affected by Feed Antibiotics in Weanling Pigs

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### Abstract

Improved understanding mode of actions of feed antibiotics in promoting growth performances in weanling pigs may contribute to the development of effective alternative dietary strategies. A total of 72 crossbred (Duroc×Yorkshire×Landrace) barrows, weaned on d 19 with an average initial body weight of 7.1 kg, were randomly assigned to two corn and soybean meal-based diets for 21 d according to a randomized complete block design. The antibiotic diet was supplemented with 550 mg aureomycin/kg. Nitrogen balance was performed on d 15 for 7 d with 9 pigs from each diet housed in individual metabolic crates for collection of total urinary excretion and fecal samples for the last 5 d. The enzyme kinetics of the jejunal and ileal aminopeptidase N (APN) activities were determined with collected jejunal and ileal samples by using L-alanine-p-nitroanilide (0-16 mM). Abundances of APN protein and mRNA were examined by quantitative real-time RT-PCR and Western blotting by using  $\beta$ -actin as a control, respectively. There were no differences ( $P>0.05$ ) in the ileal CP digestibility (control,  $67.7\pm3.7$  vs. antibiotic,  $76.3\pm1.8\%$ ) and the apparent N retention (control,  $64.8\pm0.9$  vs. antibiotic,  $65.5\pm2.4\%$ ) between the two the diets. There were no differences ( $P>0.05$ ) in the APN enzyme kinetics between the two diets. Although we had identified 14 potential N-glycosylation sites within the porcine gut APN protein catalytic pocket, the gut APN functionality was unlikely affected by N-glycosylation because of the dietary antibiotic treatment. Moreover, there were no differences ( $P>0.05$ ) in the jejunal and ileal APN mRNA and protein abundances between the two diets. Our results suggest that feed antibiotic use did not significantly improve efficiency of the digestive and post-absorptive utilization of dietary CP in promoting growth performances in the weanling pigs.

**Key words:** antibiotics; weanling pigs; dietary protein utilization; aminopeptidase N

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## The Impact of the Amino Acid Tryptophan on Behaviour, Growth and Feed Intake in Growing Pigs

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### Abstract

Aberrant behaviour in growing pigs can result in decreased growth, diminished welfare, increased morbidity and mortality and increased labour and medication costs. Tryptophan (TRP), an essential amino acid in the pig's diet, has been shown to produce calming effects in both rats and humans through its role in the serotonergic system. The objective of this study was to determine the effect of varying inclusion rates of dietary TRP on behaviour, growth and feed intake in grower pigs.

This study examined a total of 90 grower pigs divided equally across three diet treatments. The feeding trial lasted 29 days, with feed and water being fed ad libitum. A single diet was formulated based on providing all nutrients at or above their estimated NRC requirements. Amino acid mixtures were then added to the base diet to provide: 1) Control diet (100% standard ileal digestible (SID) requirement of TRP), 2) 175% SID requirement TRP, and 3) 250% SID requirement TRP. Feed intake was measured for the duration of the trial, and all pigs were weighed weekly. Pens had continuous behaviour recording for 12 hours (06:00-18:00) 3 days/week. Plasma and serum samples were taken from 3 pigs/pen (N=27pigs/trial) at four different time points (day 8, 15, 22 and 29) with TRP and serotonin levels being measured, respectively. Preliminary results indicate that the increased levels of tryptophan did not appear to have an effect on feed intake, growth rate or the prevalence of aberrant behaviour ( $P>0.05$ ), although data continue to be analyzed.

The use of supplemental TRP in pig diets may have positive implications. Producers may be able to benefit from reduced labour costs, higher growth rates and better carcass quality; the industry could benefit from an improved public perception of how pigs are raised commercially; and growing pigs may gain increased health and welfare due to decreased levels of aggression.

**Keywords:** tryptophan, behaviour, growth, feed intake

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## Increased Pre-lay Dietary Calcium up to 4.0% Does not Affect Femur Quality and Calcium Utilization in Lohmann Brown and LSL-Lite at 1<sup>st</sup> Through to the 50<sup>th</sup> Egg

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### Abstract

The effects of pre-lay dietary calcium (**Ca**) and strain on Ca utilization and femur quality at 1<sup>st</sup> through to 50<sup>th</sup> egg were evaluated using 30 Lohmann Brown (**LB**) 30 Lohmann LSL-Lite (**LSL**) pullets (14 wks of age, **woa**) reared under same management regimen. For baseline study, 6 pullets of each strain were necropsied. The rest of the pullets (24 per strain) were placed in individual cages (65 cm x 30 cm x 45 cm) and fed developer diet containing 1% Ca for 2 wks. At 16 woa, all the pullets were weighed and allocated within strains to pre-lay diets (2.5 vs. 4.0% Ca) effectively creating a 2x2 factorial arrangement (n=12). The pullets were provided pre-lay diets for 2 wk and switched to layer diet (4% Ca) at 18 woa. The diets contained TiO<sub>2</sub> to determine apparent retention (**AR**) of Ca. The age, BW and feed intake (**FI**) at 1<sup>st</sup>, 25<sup>th</sup> and 50<sup>th</sup> egg was recorded. Excreta samples were taken during pre-lay, 1<sup>st</sup> and 25<sup>th</sup> egg. Four hens per treatment were necropsied for femur samples at 1<sup>st</sup>, 25<sup>th</sup> and 50<sup>th</sup> egg. There was no interaction ( $P>0.05$ ) between pre-lay dietary Ca and strain on Ca intake, and bone mineral density (**BMD**), mineral content (**BMC**), breaking strength (**BBS**), and total ash content of femur at 1<sup>st</sup>, 25<sup>th</sup> and 50<sup>th</sup> egg. At 25<sup>th</sup> egg lay, pre-lay dietary Ca interacted with strain on BW ( $P=0.042$ ), AR of Ca ( $P=0.014$ ). The AR of Ca at pre-lay was not affected by dietary Ca ( $P=0.621$ ) but tends to by strain ( $P=0.091$ ). Pre-lay dietary Ca had no effect on BMD ( $P>0.05$ ), BMC ( $P>0.05$ ), BBS ( $P>0.05$ ) and total femur ash (TFA:  $P>0.05$ ) at 1<sup>st</sup>, 25<sup>th</sup> and 50<sup>th</sup> egg lay. The strain influenced BMD ( $P=<0.010$ ), TFA ( $P=<0.0002$ ) at 1<sup>st</sup> egg only. Hens fed increased pre-lay dietary Ca upto 4.0% did not improve bone quality parameters, however, the strain play dominant role on them.

**Keywords:** Pre-lay diet, calcium, layer, strain, bone quality

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## Determination of Standardized Ileal Digestibility of Amino Acids and Apparent Metabolizable Energy of Processed Soybean Meal (AlphaSoy) Fed to Broiler Chicks

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### Abstract

Anti-nutritional factors in soybean meal, the major source of amino acids (AA) in broiler diets, may restrict nutrient absorption in young chicks. Processed soybean meal may mitigate this challenge, however, application requires determination of digestible AA and energy for accurate feed formulation. Thus, an experiment was conducted to determine standardized (SID) ileal digestibility of AA and apparent metabolizable energy (AME) of a processed soybean product (PSBM, AlphaSoy®) in broiler chicks. A total of 180 d old male Ross 708 chicks were placed in 12 cages and fed a commercial starter diet until d 13. On d 14, birds were weighed and reallocated to one of 3 semi-purified cornstarch-based diets. The diets were a N free diet (NFD) and two 20% CP test diets formulated with either PSBM or conventional soybean meal (SBM), the same starting material for PSBM before enzyme treatment, as the sole source of N and AA. All diets had 0.5% TiO<sub>2</sub> as the indigestible marker and ratio of cornstarch to sucrose and soy oil in test diets were identical to NFD to calculate AME by difference method. Excreta samples were collected from d 17-20. On d 21, all birds were necropsied for ileal digesta. On DM basis, GE was 4481 and 4290 kcal/kg in PSBM and SBM respectively and corresponding CP values were 49.6 and 48.9%. The SID of CP was higher in PSBM (91.6 vs. 89.6%,  $P=0.04$ ). For the indispensable AA, PSBM had higher ( $P<0.05$ ) SID of His, Leu, Phe, and Val than SBM. Tendencies for higher SID of Lys (94.1 vs. 92.5%,  $P=0.06$ ) and Ile (89.3 vs. 88.3%,  $P=0.052$ ) were seen in PSBM than in SBM. The SID of Ala, Asp, Glu, Gly, Pro and Tyr were higher in PSBM than in SBM. The AME<sub>N</sub> for PSBM and SBM was 2543 and 2205 kcal/kg. These results indicated that processed soybean meal improved nutrient and energy utilization indicating improved nutritive value in broilers.

**Key words:** processed soybean meal, SID of AA, AME

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## Microalgae or Fish Oil Supplementation and Maternal Stress in Late Gestation Sows and Effects on Adrenal Gene Expression in Male Offspring

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### Abstract

Maternal stress occurring during gestation, such as microbial infection, can cause inflammation, affect programming of fetal tissues and affect offspring stress and immune responses. Maternal supplementation with omega-3 polyunsaturated fatty acids (n-3 PUFA), may minimize the effects of maternal stress on fetal development and improve offspring health. N-3 PUFA sources are mainly fish-based; microalgae (AL; *Aurantiochytrium limacinum* biomass [AURA; CCAP 4087/2] containing 70% crude fat and 17% DHA) may be an alternative. This study aimed to assess adrenal gene expression in male piglets from sows supplemented with AL or fish oil (FO) combined with a maternal stress challenge (*Escherichia coli* lipopolysaccharide, LPS). Sows were fed diets containing AL, FO or a control diet from gestation day (gd) 75. On gd 112, 8 sows per treatment were administered LPS. Seven days after weaning, 4 piglets per sow were administered LPS. After 2 hr, the piglets were euthanized, adrenal tissue was collected, and RNA was extracted for transcriptome analysis using the Affymetrix GeneChip™ Porcine Gene 1.0 ST Array. Results were analyzed using Transcriptome Analysis Console and Ingenuity Pathway Analysis, revealing enriched gene pathways relating to steroidogenesis, fatty acid metabolism and immune function. Increased expression of the immune genes MD-2, TLR-2 and NF-κB suggest that maternal AL supplementation may increase piglet sensitivity to inflammation after weaning. Decreased expression of MD-2 in offspring from sows receiving LPS suggests a role of maternal stress in reducing the offspring response to immune stress. Increased expression of 11BHSD2 in offspring from sows fed FO may also reduce the magnitude of the stress response.

**Key Words:** microalgae, fish oil, lipopolysaccharide, microarra , immune function

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# Egg Production and Egg Quality in Shaver White Layers Fed Defatted Black Soldier Fly Larvae Meal as Total Replacement of Soybean Meal from Week 28 to 43 of Age

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## Abstract

Effect of replacing soybean meal (SBM) with defatted black soldier fly larvae meal (BSFLM) in a corn-based diet fed from 28 to 43 wks of age was investigated. The sample of BSFLM (Enterra Feed Corp., BC, Canada) had concentration of 59.3, 7.0 and 6.1% DM for CP, fat and starch, respectively. A control corn-soybean meal diet was formulated to meet specifications, two additional diets were made by inclusion of either 10 or 15% BSFLM (total replacement). Diets were iso-nutritious and the target AME, CP, SID Lys, SID Met, and Ca were 2,750 kcal/kg, 15%, 0.75%, 0.45% and 4.3%, respectively and were prepared in pellet form. A total of 108, 28-wk old Shavers White hens were placed in conventional cages (6 birds/cage) and allocated diets to give 6 replicates/diet. The birds had free access to feed and water to wk 43. Egg production on cage basis was monitored daily. Feed intake and body weight was monitored in 4-wk intervals. All eggs laid on 6th d of wk 31, 35, 39 and 43 were used to measure Haugh units (HU), yolk color (YC), shell breaking strength (SBF), shell thickness (ST). There was no ( $P>0.05$ ) diet effect on HDEP, FI and HU. Egg weight and mass decreased quadratically ( $P<0.03$ ) with addition of BSFLM. Feeding BSFLM linearly increased ( $P=0.045$ ) FCR. Feeding BSFLM linearly ( $P<0.01$ ) increased yolk color intensity. A linear ( $P<0.01$ ) and quadratic ( $P=0.03$ ) increase in SBF and ST was observed with increasing BSFLM levels. In conclusion, complete replacement of soybean meal with defatted BSFLM resulted in poor FCR linked to lower egg weight suggesting some amino acids may have been limiting. Improved yolk color suggested BSFLM had pigments that increased intensity of yolk color and better shell quality indicated potential role in calcium metabolism.

**Key Words:** Black soldier fly larvae meal, Haugh Units, Egg production, shell breaking strength, shell thickness, Feed intake

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# Estimation Lysine and Phosphorus Requirements of Rainbow Trout and Nile Tilapia Along Growth Using a Factorial Approach

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## Abstract

Evidences suggest that nutrient requirements of fish changes as the fish grow. Quantifying nutrient requirement based on empirical dose-response trials is difficult since the experimental conditions, such as fish size and basal diets differ significantly between studies and which might be the cause of the wide variation for estimated values within species. Mathematical models are useful to quantify the effect of multiple factors on the nutrients requirement of fish. These models could be very valuable to help develop phase-feeding strategies and improve the cost-effectiveness of feeds.

In this study the requirements of an organic (Lys) and an inorganic nutrient (P) for a carnivorous (rainbow trout) and an omnivorous species (Nile tilapia) estimated by a factorial approach as follows:

Nutrient (Lys/P) Requirements= Retained Nutrient+ Maintenance Requirement+ Inevitable Catabolism (Lys) + Non-Fecal Losses (P)

A meta-analysis was performed on data from multiple dose-response studies. Retained nutrient estimated based on expected weight gain by integrating a growth model and body composition models. Lys intake regressed against Lys deposition. Maintenance Lys requirement estimated using an exponential model, and Lys retention efficiency (LysRE) estimated using a broken line model. Then LysRE was quantified in relation with changes in BW and feed composition. Inevitable Lys catabolism estimated as: 1- LysRE. Non-fecal P losses estimated using a mass balance approach. Optimal dietary nutrient concentration calculated from the estimated nutrient requirement (g/day) divided by the predicted feed requirement using a bioenergetics factorial model.

Results from the model simulation suggested that in both species the P requirements (expressed as a dietary concentration) decreased as fish grows, while, predicted Lys requirement slightly increases by the increase in BW. This study indicates several gaps in the availability of relevant data and the need for further investigation.

**Keywords:** Modeling, Nutrient Requirement.

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## Feeding Reduced Crude Protein, Amino Acid Balanced Diets to Laying Hens has Economic Benefits Beyond Reducing Feed Costs

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### Abstract

A study was conducted to determine if reduced crude protein (CP), amino acid (AA) balanced laying hen diets could maintain egg production and quality with concomitant reduction of production cost. Isoleucine (Ile) was previously reported to be a limiting AA in corn-SBM based layer rations that were reduced by 2 percentage units of CP from the commercial standard (16% CP). A total of 90 White Shaver hens (3 treatments, 5 hens/cage, n=6) were observed for the current analysis from 28 to 48 wks of age (woa) to determine the economic implications associated with CP reduction without (LCP) or with (LCP+Ile) supplemental Ile, which corresponded to digestible Ile to Lys ratios (dIle:dLys) of 68 and 82%, respectively. Hen day egg production, egg weight, feed conversion, and egg quality (shell strength, Haugh unit) were reported, and excreta samples were analyzed for nitrogen content. Cecal digesta was additionally analyzed for short chain and branched chain fatty acid concentrations. Nitrogen excretion was significantly ( $P<0.001$ ) lower in hens fed the LCP diets, and production performance was optimized in the LCP+Ile group. Cecal concentrations of propionic ( $P<0.01$ ) and lactic ( $P=0.02$ ) acid were higher in the LCP+Ile, and BCFA levels were numerically lower ( $P>0.1$ ) compared to the other treatments. Based on current (2019 Q1) commodity pricing in the Ontario market, the LCP diet was the least costly and the LCP+Ile diet yielded the greatest revenue, driven by increased egg production. Implementing a LCP, AA fortified diet may also save on additional production costs through improving flock health and reducing costs associated with ventilation and excreta removal.

**Keywords:** Low CP, isoleucine, laying hen, amino acids, economics

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## The Effect of Omega-3 Camelina (*Camelina Sativa*) Oil on Lipopolysaccharide Induced Osteoarthritic Cartilage Ex-plants

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### Abstract

Osteoarthritis is a debilitating joint disease in both humans and animals, causing both pain and immobility reducing welfare. This inflammatory disease is currently treated with nonsteroidal anti-inflammatory drugs, however, long term use can have negative side effects on cardiovascular and gastrointestinal health. A variety of nutraceuticals are currently on the market targeted towards treating osteoarthritis, yet there is limited research. Functional foods, such as omega-3 oils, have shown potential in reducing inflammation, with *camelina sativa* oil being used as a novel supplemental omega-3 in humans, pets and horses. The objective of this study was to test the anti-inflammatory effects of camelina oil on a cartilage ex-plant model of osteoarthritis. Current results show camelina oil reduces prostaglandin E2 in healthy cartilage, but not in lipopolysaccharide induced arthritic cartilage. Analysis of nitric oxide, glycosaminoglycan and cell viability are ongoing. If these results stay consistent, camelina oil could prove to be a viable alternative to preventing osteoarthritis.

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## The Effects of Supplementing Commercially Available Feed Additives (CFA) to Nursery Pig Diets Contaminated with Deoxynivalenol (DON)

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### Abstract

In North America, deoxynivalenol (DON) is a prevalent mycotoxin in cereal grains. Feeding DON contaminated feed reduces performance and profitability in swine production. Supplementing feed additives is one of the possible methods to reduce negative impact of mycotoxins, however, results are controversial. The purpose of this study was to evaluate the effects of four different commercially available feed additives when added to DON contaminated feed, on growth performance and physiology of nursery pigs when fed for 4-wk. Total of 144 nursery pigs (IBW 9.8 0.5kg; 2 gilts, 2 barrows/pen) were used. Six test corn-soybean-meal based diets were: 1) positive control (PC), formulated with clean corn (<1 ppm DON), 2) negative control (NC), formulated with contaminated corn (5.5 ppm DON), 3) NC with enzyme+binder1 (NCB1), 4) NC with clay (NCC), 5) NC with enzyme+binder2 (NCB2) and 6) NC with sodium-metabisulfite (NCP). Diets were randomly allocated to pens (n=6) based on BW. Weekly BW and FI were monitored. At the end of wk-1, one pig/pen was euthanized for tissue collection. Concentration of DON in PC, NC, NCB1, NCC, NCB2 and NCP were 0.38, 2.3, 2.3, 2.3, 2.4, and 1.9 ppm, respectively. Feed-intake was not affected ( $P>0.10$ ) by treatments throughout the study. In wk-1, piglets fed NCP had higher ( $P\leq 0.03$ ) ADG and G:F compared with piglets fed NC and NCC diets while other treatments were not differ with any treatment. No differences ( $P>0.10$ ) in organ weights, jejunum morphology and plasma concentration of creatine and urea were shown. Lactic, acetic, propionic and n-butyric acid concentrations in cecal digesta was not different among treatments however, citric acid was highest ( $P=0.03$ ) in NCP relative to NCB2. In conclusion, NCP improved ADG and G:F only in wk-1 with no differences on physiological responses.

Key words: DON, feed additives, mycotoxin, nursery pigs

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## Ability of Eubiotics and an Enzyme Blend to Replace Antibiotic Growth Promoters in Commercial Broilers

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### Abstract

The poultry industry needs alternatives to antibiotic growth promoters (AGP). The effect of a combination of eubiotics (additives that promote a healthy gastrointestinal microbiota) and a mixture of enzymes as an alternative to AGP in broiler diets was tested in a large-scale commercial field trial (2,066,000 broilers). 56 environmentally-controlled barns (experimental unit) were distributed across 6 farms in Guayas, Ecuador in a completely randomized design. Pre-starter (0-7d); starter (8-20d); grower (21-35d); and finisher (36d-end of cycle) diets included corn, soybean meal, oats, and rice bran in mash form. The basal diet included *Enterococcus faecium*, essential oils, xylanase, amylase, and protease. Treatments were: Control (T1): AGP + butyrate + prebiotic + 1,000 FYT/Kg phytase; T2: T1 without AGP + 2,500 FYT/Kg phytase + *Bacillus subtilis* and *Bacillus licheniformis*; T3: T2 without butyrate and prebiotic. Performance, mortality, carcass yield, footpad lesion score, intestinal integrity index, ileal and cecal bacterial abundance (Enterobacteriaceae (EB), Clostridiales (CT), and Lactobacillus (LB), litter moisture, and total nitrogen were measured. Data were analyzed using one-way ANOVA with a P<0.05 level of significance. There were no effects of treatment on performance (P> 0.1). Mortality tended (P= 0.1052) to be lower in T3 (2.76% ± 0.160%) compared to T1 (3.26% ± 0.165%). There were no differences between the treatments on any of the performance, health or footpad health welfare, bacterial abundance at ileum or cecum for LB and EB, nor litter measurements (P> 0.1). Cecal CT counts were not affected, but ileal counts in T2 and T3 were lower (P= 0.0167, ± 0.103 log CFU/g). Because there was no detrimental effect of T2 or T3 in this large-scale commercial trial, it is feasible to replace AGP with one of these strategies without reducing bird health or productive performance.

**Key words:** Broilers, enzymes, AGP, eubiotics, field trial

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## Interactive Effects of Fibrous Feed Ingredients and Multi-enzyme Supplement: Comparative Growth Performance in Broiler Chicks and Turkey Poults

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### Abstract

We investigated interactive effects of grain (corn or wheat), fiber (low or high), and multi-enzyme supplement (MES) in broiler chicks (Exp 1) and turkey poults (Exp 2). High fiber diets were created by adding corn DDGS and wheat middlings at 10% in corn and wheat-based diets, respectively. The MES supplied 2,500 U of xylanase, 300 U of  $\beta$ -glucanase, 700 U invertase, 10,000 U of protease, 1,200 U of cellulase, 24,000 U of amylase and 20 U of mannanase per kg of feed, respectively. All the diets met or exceeded specifications and were prepared in pellet form. A total of 960-d old Ross 708 chicks (Exp 1) and 720-d old hybrid toms (Exp 2) were allocated to eight diets (n=6). Birds had free access to feed and water for 28 days. Body weight and feed intake were recorded. There were no ( $P>0.10$ ) treatment effects on feed intake and feed conversion in both experiments. In Exp 1, an interaction ( $P<0.05$ ) between grain, fiber and MES, was such that corn diets with high fiber reduced FBW and BWG but MES addition improved these parameters by 3.5%; additionally, broilers fed high fiber wheat diets and MES had a lower FBW and BWG compared to birds fed all other diets. Broilers fed corn had a higher FBW (1462 vs. 1424) and BWG (1416 vs. 1378) than birds fed wheat diets ( $P<0.05$ ). In Exp 2, there was no interaction ( $P>0.1$ ) between grain, fiber and MES or main effects of MES on FBW and BWG. Poults fed wheat had a higher ( $P<0.05$ ) FBW (1141 vs. 1408) and BWG (1376 vs. 1343) than poults fed corn diets. In conclusions, wheat-based diets increased FBW and BWG in poults but reduced them in broiler chickens. Fiber in corn diets reduced growth in broilers which was improved by MES.

**Keywords:** broiler, turkey, fiber, growth performance, multi-enzyme supplement

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## The Effects of Steam Explosion, Die Specification, and Fibre Source on Pellet Quality and Fibre Composition

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### Abstract

Locally sourced, fibrous co-products play a significant role in ration formulations throughout western Canada. Processing allows for higher inclusion of co-products and densifies the product saving on transport costs and reducing waste when feeding. This study was conducted to examine the effect of pellet die specification and preprocessing techniques on pellet quality and nutrient composition. Trial one evaluated complete diets formulated with two different sources of fibrous co-products, (oat hulls vs. pea hulls) included at 20.5 % of the mixture, and four die size/compression ratio combinations, (4mm/12.5; 8mm/9; 8 mm/11.56; 11mm/8.41). Identical production runs were conducted in triplicate for each co-product alternating the fibre source on each run. In trial two the two fibrous co-products were preprocessed using steam explosion prior to inclusion in the same diets as trial one and pelleted (4mm/12.5) in triplicate runs. . In all production runs, samples were collected at mixing, conditioning, pelleting and cooling for determination of pellet durability index, (**PDI**), density, NDF and ADF. Diets formulated with pea hulls demonstrated a greater **PDI** ( $P < 0.05$ ) and density ( $P < 0.05$ ) compared with diets formulated with oat hulls. Die specifications did not influence pellet characteristics. Steam explosion preprocessing of both fibre co-products lowered the NDF ( $P < 0.05$ ) and ADF ( $P < 0.05$ ) concentrations but a reduction of lignin only in the oat hulls ( $P < 0.05$ ). In conclusion, diets containing pea hulls resulted in a more durable and denser pellet, whereas diets containing oat hulls improved pellet hardness. Steam explosion modified fibre composition, potentially enhancing the nutritional value without affecting pellet characteristics. Further work is required to determine animal performance and thus economic benefit.

**Key words:** NDF, ADF, steam explosion, die specification, oat hulls, pea hulls

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## Effect of Red Seaweed (*Chondrus crispus*) on Feed Conversion in Heat Stressed Laying Hens

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### Abstract

Seaweed is considered a prebiotic with beneficial micronutrients that have shown to positively influence gut function and performance in laying hens. The purpose of this study was to monitor production performance of heat stressed laying hens fed *Chondrus crispus* (CC). The experiment was a 2 x 3 x 2 x 2 factorial in a completely randomized design with processing method of the CC [Ground and Extruded], inclusion level [0, 0.5, and 3%], strain of hen [Lohmann Lite-LSL White (LL) and Lohmann Lite Brown (LB)] and heat challenge [Heat and No Heat] as the main effects. Birds kept in the heat stressed environment were challenged with rising heat levels from 11AM to 6PM, where temperatures gradually rose from 23-24°C to 33°C. At 6pm, the temperature gradually dropped back to 23-24°C. A total of 256 birds were used, with 8 replicates per treatment combination (2 birds per cage). Feed consumption and eggs produced was recorded daily. At the end of the trial, feeders were weighed and 2 eggs per cage were measured for egg weight. Results were analyzed as a factorial arrangement using the Proc Mixed Procedure of ANOVA. LB hens consumed significantly more ( $P<0.05$ ) feed (115.93g/day) than LL hens (112.16g/day). For average egg weight, there was an interaction effect ( $P<0.05$ ) between processing and inclusion level whereby hens fed ground CC had significantly larger eggs at the 3% level (64.335g) compared to the 0% level (62.185g). For feed conversion, an interaction effect ( $P<0.05$ ) was observed between strain and heat treatment whereby LB birds had more efficient feed conversion when challenged with heat (1.956) compared to LB birds kept in regular temperatures (2.124). LL birds demonstrated significantly ( $P<0.05$ ) superior feed conversion in the non-heat environment (1.834) compared to LB in both heat and non-heat environments. Thus, inclusion of ground CC resulted in increased egg weights, while LL birds demonstrated significantly improved feed conversion compared to LB when heat challenged, regardless of CC.

**KEYWORDS:** Seaweed, laying hen, FCR

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## Impact of Dietary Fibre and Indigestible Protein on Threonine Requirements for Growth and Gut Health of Growing Pigs

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### Abstract

Co-products are increasingly being used in swine diets resulting in greater content of dietary fibre (DF) and indigestible protein (IP) which can both impact gut health and nutrient requirements. A nitrogen (N)-balance study was conducted to determine the impact of DF and IP on threonine (Thr) requirements for N-retention and indicators of gut health. A total of 160 pigs at  $19.4 \pm 1.05$  kg were randomly assigned to 1 of 20 dietary treatments ( $n=8/\text{trt}$ ) on a  $2 \times 2 \times 5$  factorial design with DF (high and low), IP (high and low) and Thr (0.52, 0.60, 0.68, 0.76, and 0.82% SID) as factors. High DF was achieved by the addition of 10% sugar beet pulp and 5% wheat bran. Dietary IP was included as heat-damaged soybean meal. An 8d adaptation period was followed by a 4d N balance collection period. On d 13, pigs fed the 0.52, 0.68, and 0.82% Thr diets were euthanized for collection of tissue and digesta samples from colon for analysis of mucin (MUC2) and tight junction (CLDN4, ZO1) gene expression and ammonia concentration. All data were analyzed by PROC MIXED with fixed effects of DF, IP, Thr, and their interactions, with block as a random effect. Thr requirements were estimated using PROC NLIN quadratic curvilinear break-point model. The Thr requirement for maximum N-balance was estimated at 0.68, 0.64, 0.72, and 0.68% SID for Low DF-Low IP, Low DF-High IP, High DF-Low IP, and High DF-High IP fed pigs, respectively, indicating an increased Thr requirement in high DF diets and decreased requirement with greater IP. High IP increased ( $P<0.05$ ) and high DF decreased ( $P<0.05$ ) and decreased ( $P<0.05$ ) digesta ammonia concentration. Both high DF and high IP independently increased MUC2, CLDN4, and ZO1 expression which were reduced when both high DF and IP were present ( $P<0.05$ ). Overall these results demonstrate that DF and IP impact Thr requirements for N-retention, however, the interaction between the two factors requires further research.

Key words: fermentable fibre, indigestible protein, swine, gut health, amino acids, threonine

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## Intestinal Responses and the Determination of True Total Tract Trace Mineral Digestibility in Weanling Pigs by the Regression Analysis Technique

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### Abstract

There is a scarcity of information available regarding trace mineral bioavailability in trace mineral supplements and common feed ingredients to guide swine diet formulation. Twenty-four crossbred weanling barrows, with an average initial body weight (BW) of 14 kg, were randomly assigned to 4 weanling diets and were fed close to *ad libitum* for 11 d according to a randomized complete block design. The 4 diets were corn, soybean meal and dried whey powder based and were formulated with inclusion of a commercial trace mineral-vitamin premix at 4 gradient levels (at 0.125, 0.250, 0.375 and 0.500%, respectively) to result in 4 graded levels of trace minerals of Zn, Cu, Fe and Mn. Titanium dioxide (0.30%) was included in the diets as a digestibility marker. The gut permeability marker of D-mannitol was fed 0.30 g/kg BW at 4 h before the pigs were sacrificed for blood and tissue sampling. The dietary inclusion of 4 gradient levels of the commercial trace mineral-vitamin premix did not affect ( $P>0.05$ ) growth performances, the apparent ileal and fecal dry matter digestibility, gut permeability and jejunal alkaline phosphatase activity kinetics. With the regression analysis technique, true total tract Cu, Mn, Fe and Zn digestibility ( $\pm$ SE) in the commercial trace mineral-vitamin supplement premix was determined to be  $137.3\pm27.9$ ,  $94.3\pm21.6$ ,  $66.2\pm24.6$  and  $40.5\pm15.4\%$  ( $n=24$ ), respectively. The true total tract trace mineral digestibility in the four weanling pig compound diets was very high for Cu at 137.4% (SEM=5.0,  $n=6$ ) and Mn at 94.2% (SEM=5.7,  $n=6$ ), intermediate for Fe at 66.2% (SEM=5.1,  $n=6$ ), and was relatively low for Zn at 40.4% (SEM=4.9,  $n=6$ ). Swine diets should be formulated on the basis of true total tract digestible trace mineral supply to reduce feeding cost and detrimental impacts of excessive manure heavy trace minerals on the environment.

**Key words:** trace minerals; true total tract digestibility; the regression analysis; weanling pigs

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