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2020 COLLOQUE DE NUTRITION
ANIMALE DU CANADA

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26-28
May/mai 2020

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June/juin 2020

Exploring the links between animal nutrition and animal health

Explorer le lien entre la nutrition et la santé animale

ANAC Welcome

I am pleased to welcome you to the virtual edition of the 2020 Animal Nutrition Conference of Canada (ANCC). Although we cannot be together in person this year, now more than ever, it is critical that we share knowledge and new scientific developments so we can continue to feed the world's food-producing animals.

As animal nutrition professionals, we are well-accustomed to adapting to challenging conditions including increasing pressure to reduce the use of traditional antibiotics in animal production, while maintaining high performing animals who derive affordable protein for human consumption. And, in these COVID-19 times, we find ourselves using our nutritional expertise to assist our customers in adapting production practices as they navigate unprecedented processing capacity and financial challenges. Whether you can participate in one or all six conference sessions, we hope the learnings you take away over the next few weeks spark further conversations and innovations that can be applied to current and future animal nutrition challenges.

An event of this magnitude is not possible without the generous contributions of our many industry partners. We thank our sponsors who have shown incredible support for the conference throughout and have enabled us to offer this online learning opportunity at no cost to participants. We extend our appreciation as well to our dedicated speakers for sharing their expertise. The virtual format has allowed us to bring the exciting research of our leading scientists and students to a record number of participants from around the globe.

The organizing committee has once again succeeded in putting together an exceptional program. In March, when final details of the conference were being prepared for Winnipeg, the committee was asked to pivot and plan for a completely new format. We would like to recognize all organizing committee members for their hard work, responsiveness and dedication to the success of ANCC 2020.

Enjoy the series of webinars, stay safe, and we hope to meet you in person next year in Montreal!

Melissa Dumont, agr.
Executive Director, Animal Nutrition Association of Canada

Mot de bienvenue de l'ANAC

Je suis heureuse de vous accueillir à l'édition virtuelle du Colloque de nutrition animale du Canada (CNAC) de 2020. Bien que nous ne puissions pas être ensemble en personne cette année, il est plus que jamais essentiel que nous partagions nos connaissances et les nouveaux développements scientifiques afin de continuer à nourrir les animaux producteurs d'aliments dans le monde.

En tant que professionnels de la nutrition animale, nous sommes bien habitués à nous adapter à des conditions difficiles, notamment à la pression croissante visant à réduire l'utilisation des antibiotiques traditionnels dans la production animale, tout en maintenant des animaux performants qui produisent des protéines à prix abordable pour la consommation humaine. Et, en cette période de COVID-19, nous nous retrouvons à utiliser notre expertise nutritionnelle pour aider nos clients à adapter leurs pratiques de production alors qu'ils doivent faire face à une diminution de capacités d'abatages et de transformations et à des défis financiers sans précédent. Que vous puissiez participer à une seule ou aux six sessions du colloque, nous espérons que les enseignements que vous en retirerez au cours des prochaines semaines susciteront d'autres conversations et innovations pouvant être appliquées aux défis actuels et futurs en matière de nutrition animale.

Un événement de cette ampleur n'est pas possible sans la généreuse contribution de nos nombreux partenaires de l'industrie. Nous remercions nos commanditaires qui ont apporté un soutien incroyable au colloque au fil des ans et nous ont permis, cette année, d'offrir gratuitement cette opportunité d'apprentissage en ligne aux participants. Nous remercions également nos conférenciers dévoués qui ont accepté de partager leur expertise. Le format virtuel nous permettra de présenter les recherches passionnantes de nos scientifiques et étudiants à un nombre record de participants du monde entier.

Le Comité organisateur a une fois de plus réussi à mettre sur pied un programme exceptionnel. En mars, lorsque les derniers détails du colloque étaient mis au point en prévision de notre rencontre à Winnipeg, nous avons demandé au comité de pivoter et de planifier un format complètement nouveau. Nous tenons à remercier tous les membres du Comité organisateur pour leur travail acharné, l'adaptabilité et le dévouement dont ils ont fait preuve pour contribuer au succès du CNAC 2020.

Profitez de la série de webinaires, restez en sécurité, et au plaisir de vous rencontrer en personne l'année prochaine à Montréal!

Melissa Dumont, agr.
Directrice exécutive, Association de nutrition animale du Canada

ANCC 2020 Organizing Committee / Comité organisateur du CNAC 2020

We are honoured to welcome you to the 4th annual Animal Nutrition Conference of Canada (ANCC). This year's conference enters uncharted territory for the ANCC, with seminars delivered in digital format to accommodate social distancing measures required during this challenging time. The ANCC continues its tradition of bringing 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, "Exploring the Links Between Animal Nutrition and Animal Health". This timely theme explores topics such as macronutrient and micronutrient influences on gut function, microbiota and host interactions for optimal health, and nutritional influences on immune development. We investigate the role that nutrition plays on maintaining healthy, productive livestock raised without the use of antibiotics. We examine how animal nutrition programs influence sustainable production systems, and discuss the connection between healthy livestock and healthy people.

In building the program for ANCC 2020, the committee sought to display innovative Canadian research while tapping into scientific expertise from around the globe. We are eager to highlight novel research from Canadian students and from our industry partners. We are thankful to the Animal Nutrition Association of Canada and the ANCC 2020 sponsors for supporting this unique occasion to connect and share knowledge. We hope that our participants will appreciate the opportunity to gain insight into strategies that may be employed in their daily work to promote healthy, sustainable livestock and poultry production. Take care and enjoy the conference!

Nous sommes honorés de vous accueillir au 4e Colloque de nutrition animale du Canada (CNAC). Cet événement s'aventure cette année, présentant des séminaires en format numérique pour tenir compte des mesures de distanciation sociale requises en cette période difficile. Le CNAC poursuit sa tradition de réunir des chercheurs et des spécialistes de l'industrie de l'alimentation animale pour un échange dynamique de connaissances sur les derniers développements scientifiques en matière de nutrition du bétail et de la volaille. Cette année, nous avons choisi le thème « Explorer les liens entre l'alimentation et la santé animales ». Ce thème d'actualité explore des sujets tels que l'influence des macronutriments et des micronutriments sur la fonction intestinale, les interactions entre le microbiote et l'hôte pour une santé optimale et l'influence des nutriments sur le développement immunitaire. Nous étudions le rôle que joue la nutrition dans le maintien d'un bétail sain et productif, élevé sans antibiotiques. Nous examinons comment les programmes de nutrition animale influencent les systèmes de production durables et discutons du lien entre un bétail en bonne santé et des personnes en bonne santé.

En élaborant le programme du CNAC 2020, le comité a cherché à présenter les recherches canadiennes innovantes tout en faisant appel à l'expertise scientifique du monde entier. Nous sommes impatients de mettre en valeur les recherches novatrices des étudiants canadiens et de nos partenaires de l'industrie. Nous sommes reconnaissants à l'Association de nutrition animale du Canada et aux commanditaires du CNAC 2020 d'avoir soutenu cette occasion unique de se connecter et de partager des connaissances. Nous espérons que nos participants apprécieront l'occasion qui leur est donnée de se faire une idée des stratégies qui peuvent être utilisées dans leur travail quotidien pour promouvoir une production animale et avicole saine et durable. Prenez soin de vous et tirez le maximum du colloque!

Amy Johnston, Manitoba Agriculture
(Program Chair / Présidente du programme du colloque)

Anna Rogiewicz, University of Manitoba
(Academic Chair / Présidente de l'académie)

Theunis Wessels, Lallemand Animal Nutrition
(Sponsorship Chair / Président du parrainage)

Jeff Bond, PMT Inc.

Annick Delaquis, Sollio Agriculture

Jeff Keunen, Grand Valley Fortifiers

Emma McGeough, University of Manitoba

Holly McGill, Wallenstein Feed & Supply Ltd.

Jeffrey Park, ADM Animal Nutrition

Rob Patterson, Canadian Bio-Systems Inc.

Kayla Price, Alltech Canada

Sarah Hopkins, My-Lien Bosch, Nancy Stonos-Smith, Josée Lafontaine (ANAC)

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TUESDAY, MAY 26TH – GENERAL / *MARDI 26 MAI – GÉNÉRAL*

1	Opening Remarks / *Propos d'ouverture*	
2	**JM Bell Lectureship in Animal Nutrition**: Influencing microbiota-host interactions by dietary strategies to improve intestinal health ***Conférence JM Bell en nutrition animale*** *: Influencer les interactions microbiote-hôte pour améliorer la santé intestinale par des stratégies alimentaires*	**Dr. Filip Van Immerseel** Ghent University UNIVERSITY OF SASKATCHEWAN
3	**ANAC Scholarship Recipient :** Effects of lipid inclusion and saturation of dietary fatty acids on nutrient transport across the ruminant gastrointestinal tract ***Récipiendaire de la bourse d'études de l'ANAC :*** *Effets de l'inclusion de lipides et de la saturation des acides gras alimentaires sur le transport des nutriments dans le tractus gastro-intestinal des ruminants*	**Liam Kelln** University of Saskatchewan
4	The impact of minerals on feed stability and the antioxidant pathway *L'impact des minéraux sur la stabilité des aliments et la voie des antioxydants*	**Dr. Richard Murphy*** Alltech **Alltech®**
	For an opportunity to speak further with **Dr. Richard Murphy** please contact Jaime Cutmore at Jaime.Cutmore@Alltech.com to schedule an appointment.	

* Pre-Conference Speaker / *Conférencier précolloque*

THURSDAY, MAY 28ᵀᴴ - RUMINANT/ *JEUDI 28 MAI – LES RUMINANTS*

1	Supporting healthy immune function in transition dairy cows *Soutenir une fonction immunitaire saine chez les vaches laitières en transition*	**Dr. Stephen LeBlanc** University of Guelph
2	Nutrient absorption and health in young calves *Absorption des nutriments et santé des jeunes veaux*	**Dr. Anne Laarman** University of Alberta
3	From human research to animal research and application: How do we make the leap? *De la recherche humaine à la recherche animale et son application : comment faire le saut?*	**Dr. Dana Tomlinson*** Zinpro ZINPRO
	Dr. Dana Tomlinson will remain online and be available for further discussion. If you would like to participate in this extended session please remain logged into the webinar.	

TUESDAY, JUNE 2ᴺᴰ – MONOGASTRIC / *MARDI 2 JUIN – LES MONOGASTRIQUES*

1	Feeding pigs and broilers for their health: The need to go beyond animal performance - from a meta-analytic approach to animal trials *Nourrir les porcs et les poulets de chair pour leur santé : la nécessité d'aller au-delà de la performance animale - d'une approche méta-analytique à l'expérimentation animale*	**Dr. Marie-Pierre Létourneau-Montminy** Université Laval
2	Immunometabolism: The potential cause of and solution to our most pressing poultry problems in health and infectious disease *Immunométabolisme : la cause potentielle et la solution de nos problèmes avicoles les plus urgents en matière de santé et de maladies infectieuses*	**Dr. Ryan Arsenault** University of Delaware
3	Diet complexity and L-threonine supplementation: effects on growth performance, immune response, and intestinal integrity in nursery pigs *Complexité du régime alimentaire et supplémentation en L-thréonine : effets sur la performance de croissance, la réponse immunitaire et l'intégrité intestinale chez les porcs en pouponnière*	**Bonjin Koo** University of Manitoba Graduate Student / *Étudiant diplômé*
4	The interaction of dietary protein on gut health, microbiota and animal performance *L'interaction des protéines alimentaires sur la santé intestinale, le microbiote et la performance animale*	**Dr. Elizabeth Santin*** Jefo Jefo
	Dr. Elizabeth Santin will remain online and be available for further discussion. If you would like to participate in this extended session please remain logged into the webinar.	

* Pre-Conference Speaker / *Conférencier précolloque*

THURSDAY, JUNE 4TH - RUMINANT/ *JEUDI 4 JUIN – LES RUMINANTS*

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2	Diet for healthy gut: the secret for overall health and productivity of dairy cattle *L'alimentation pour une bonne santé intestinale : le secret de la santé et de la productivité globales des bovins laitiers*	**Dr. Renée Petri** Agriculture and Agri-Food Canada
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	Dr. Christos Gougoulias will remain online and be available for further discussion. If you would like to participate in this extended session please remain logged into the webinar.	

* Pre-Conference Speaker / *Conférencier précolloque*

TUESDAY, JUNE 9TH – MONOGASTRIC & GENERAL /
MARDI 9 JUIN – LES MONOGASTRIQUES & GÉNÉRAL

1	Non-antibiotic strategies to reduce inflammation in poultry *Stratégies non antibiotiques pour réduire l'inflammation chez les volailles*	**Dr. Doug Korver** University of Alberta
2	Feed processing for optimal animal health and performance *Transformation des aliments et optimisation de la santé et du rendement des animaux*	**Dr. Rex Newkirk** University of Saskatchewan
3	Bacterial debris – Peptidoglycans and their influence on gut functionality *Débris bactériens - les peptidoglycanes et leur influence sur la fonctionnalité intestinale*	**Dr. Mikkel Klausen*** Novozymes DSM
	Dr. Mikkel Klausen will remain online and be available for further discussion. If you would like to participate in this extended session please remain logged into the webinar.	

THURSDAY, JUNE 11TH – RUMINANT & GENERAL /
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1	The role of livestock in sustainable production systems in Canada *Le rôle du bétail dans les systèmes de production durables au Canada*	**Dr. Kim Ominski** University of Manitoba
2	The impact of animal products on human health: A 2020 vision of the evidence *L'impact des produits d'origine animale sur la santé humaine : une vision 2020 des preuves scientifiques*	**Dr. Daniel Rico** Centre de recherche en sciences animales de Deschambault (CRSAD)
3	Closing remarks / Propos de clôture	

* Pre-Conference Speaker / *Conférencier précolloque*

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References: García-Rebollar et al., 2016 | Lagos and H. H. Stein, 2017 | Mateos, 2018
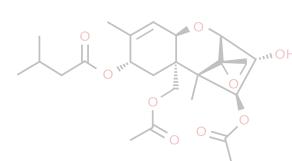
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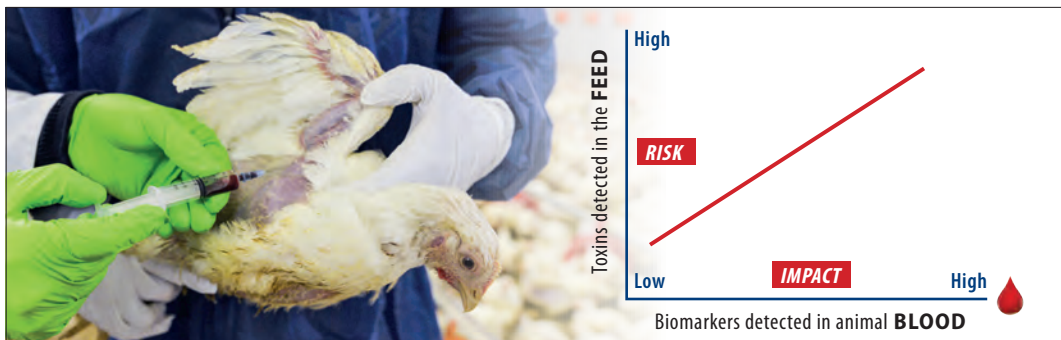
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Dr. Christos Gougoulias
Innovad SA, Postbaan 69, 2910 Essen, Belgium
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Abstract

Mycotoxins in feed and relevant crop (field) surveys have been major focuses within livestock production until today. However, the lack of evaluation of the true impact of detoxifier products on systemic levels of mycotoxins in animals and possible correlation to their health status is evident.

We here report significant systemic reduction of aflatoxin B1 (AFB1-40%) and deoxynivalenol (DON-50%) in broiler plasma, under concomitant administration of high concentrations of a mycotoxin blend [DON at 5 mg/kg feed i.e. the maximum permitted level by the European Food Safety Authority (EFSA), AFB1 at 20 mg/kg feed i.e. 1000x higher, and ochratoxin A (OTA) at 2.5 mg/kg feed i.e. 25x higher than the maximum safe EFSA guidelines] and detoxifier dose (2.4g/kg feed) studied. A similar trend of systemic reduction of DON-Glc-A in plasma and DON in urine was seen in pigs, under concomitant administration of DON (0.9 mg/kg feed i.e. the maximum permitted level by EFSA) and zearalenone (ZEN at 75 mg/kg feed i.e. 750x higher than the maximum safe EFSA guidelines) suggesting thus, significant beneficial bioactivity. Additionally, we report the successful transfer of the analytical methodology from plasma to dried blood spots (r>0.947), whereby a drop of blood is collected on a filter paper. Advantages include the sampling itself, reduced animal invasiveness, transportation and increased storage. This has now been transferred in real field conditions with a novel service 'Myco-marker®'. This supportive diagnostic tool assesses, both in feed &

blood, the overall impact of mycotoxins on animal health and performance with high sensitivity (LC-MS/MS).

Résumé

Les mycotoxines dans les aliments du bétail et les analyses de cultures pertinentes ont été les principaux centres d'intérêt en production animale jusqu'à aujourd'hui. Toutefois, on constate une absence d'évaluation de l'impact réel des produits détoxifiants sur les concentrations systémiques de mycotoxines chez les animaux et de la corrélation possible avec la santé.

Nous avons observé une réduction systémique significative des concentrations d'aflatoxine B1 (AFB1-40 %) et de désoxynivalénol (DON-50 %) dans le plasma des poulets à griller après administration concomitante de fortes concentrations d'un mélange de mycotoxines [DON à 5 mg/kg d'aliments, soit la teneur maximale autorisée par l'Autorité européenne de sécurité des aliments [EFSA], AFB1 à 20 mg/kg d'aliments, soit 1000 fois plus, et ochratoxine A [OTA] à 2,5 mg/kg d'aliments, soit 25 fois plus que les recommandations maximales de sécurité de l'EFSA) et d'une dose du détoxifiant (2,4 g/kg d'aliments) étudié. Une tendance similaire en faveur d'une réduction systémique de DON-Glc-A dans le plasma et de DON dans les urines a été observée chez les porcs à la suite de l'administration concomitante de DON (0,9 mg/kg d'aliments, soit la dose maximale autorisée par l'EFSA) et de zéaralénone (ZEN à 75 mg/kg d'aliments, soit 750 fois plus que les recommandations maximales de sécurité de l'EFSA), ce qui suggère une bioactivité bénéfique significative. En outre, nous annonçons le transfert réussi de la méthode de l'analyse du plasma vers celle de l'analyse des gouttes de sang séché (r>0,947), par laquelle une goutte de sang est recueillie sur un papier filtre. Il s'agit à la fois d'une méthode d'échantillonnage plus simple à réaliser et moins invasive pour l'animal, et les échantillons recueillis sont plus faciles à transporter et à conserver. Cette technique est maintenant utilisée dans des conditions réelles sur le terrain par le biais d'un nouveau service, « Myco-marker® ». Cet outil de diagnostic complémentaire permet d'évaluer, à la fois dans les aliments et le sang, l'impact global des mycotoxines sur la santé et les performances des animaux avec une grande précision (LC-MS/MS).

Introduction

Globally, the ever-increasing demand for protein consumption (via meat production) in a sustainable manner, requires further reduction of the overall production cost. Feed cost remains key to this. However, variability of raw material costs and the complex load of mycotoxins contamination continue to negatively impact productivity.

On top of that, animal health and productivity are affected by a number of other stress factors including low quality of feed, diet composition changes, heat, vaccinations, infections etc. In addition, modern farming animals possess limited natural resistance and immunity against such stresses. Mycotoxins worsen the impact of such stress factors and tend to impair key metabolic organs such as the liver and kidneys. The negative impact of mycotoxins expands to defence mechanisms and the immune system. For example, DON and fumonisins increase the paracellular gut permeability and predispose to the development of *Clostridium perfringens*-induced necrotic enteritis in broilers (Antonissen et al. 2014; Antonissen et al. 2015) whereas, DON in pigs inhibits vaccination efficiency of PRRSV live vaccines (Savard, Gagnon, and Chorfi 2015).

Presence of mycotoxins in grain is widespread and it is estimated that ~90% of samples contain mycotoxins and nearly 40% contain multiple mycotoxins, which can exert a synergistic negative effect to animals (Kovalsky et al. 2016; Eskola et al. 2019).

For appropriate feed risk assessment, several mycotoxins and their fungal and plant metabolites need to be quantified with high precision. In that respect, quick and often cheap analyses tools such as Lateral Flow Devices (LFDs) and ELISAs are considered incomplete approaches, when used in isolation. The problematic sampling of raw materials with the known 'hot spots' adds dramatically to this distorted picture. More importantly, inherently, the feed risk assessment approach *per se*, does not provide a true representation of the true exposure of animals to mycotoxins.

Therefore, the primary aims here were: a) the development and validation of biomarkers of exposure i.e. several mycotoxins and their Phase I and Phase II metabolites, detected with high analytical approaches (LC-MS/MS and HRMS), b) in different biological fluids in broiler chickens and pigs, c) the application of these biomarkers of exposure as a tool for testing the efficacy of a mycotoxin detoxifier (Escent® S) in broilers and pigs and, d) the transfer of the validated methodology into a practical, user-friendly as well as easy to sample and transport system, namely in dried blood spots (DBS).

Results

Chicken
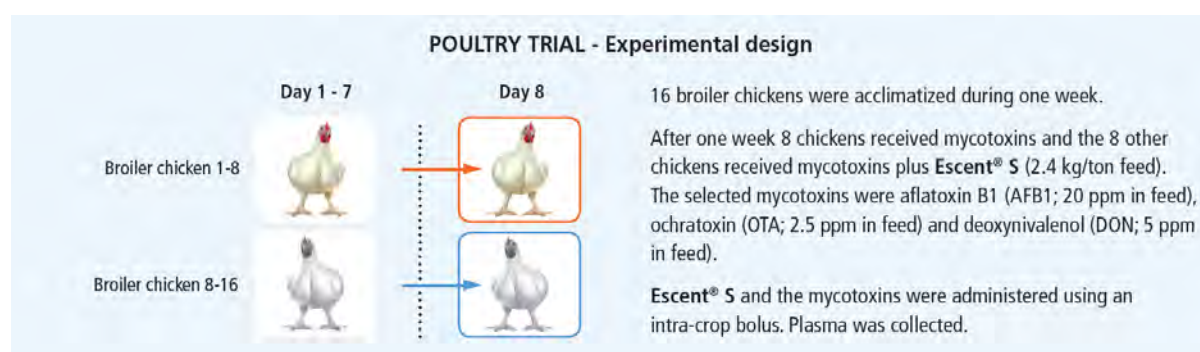
Fig. 1 Study design of the trials in broilers indicating number of animals per treatment, dose of detoxifier and dose of multiple mycotoxins.
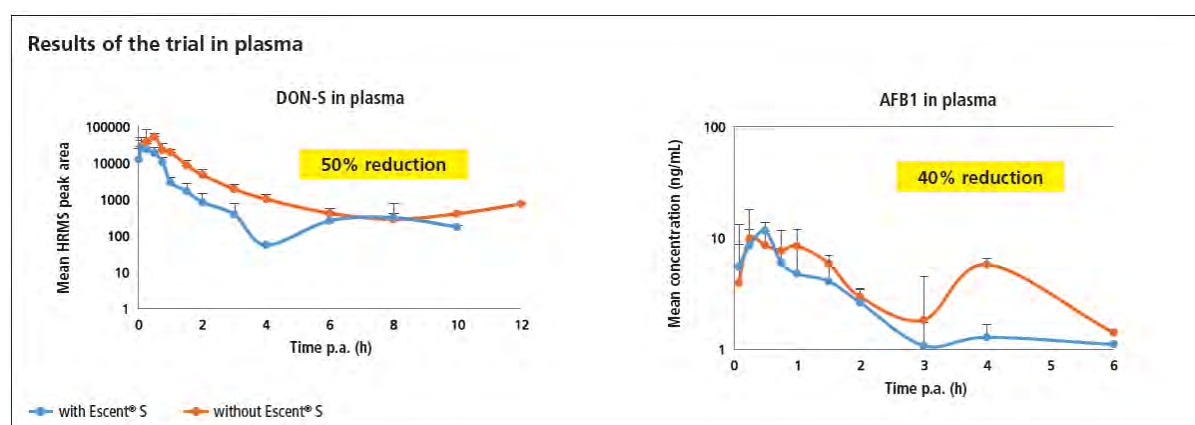
Fig. 2 Mean HRMS peak area-time curves (+ SD) of deoxynivalenol-sulphate (DON-S (a) and mean concentration-time curves (+ SD) of AFB1 (b) in plasma of broiler chickens after oral administration of a bolus of DON (0.5 mg/kg BW), OTA (0.25 mg/kg BW) and AFB1 (2 mg/kg BW), either with Escent® S (treatment group, n=8, blue curve) or without (control group, n=8, orange curve).

The detoxifier technology Escent® S showed a statistically significant positive effect on chicken systemic detoxification compared to the control treatment, when the mean percentage difference of the corresponding areas under the concentration-time curve were calculated ($p<0.05$). Namely, it was shown that the Escent® S treatment reduced by 50% the exposure of DON and by 40% the exposure to AFB1 (Aflatoxin B1) in chicken blood (Lauwers, Croubels, Letor, et al. 2019). It should be noted that the animals were contaminated with a blend of three mycotoxins (DON, OTA and AFB1) with the levels of OTA being 25 times higher and AFB1 1,000 times higher than the EFSA guidelines. The findings are of great importance taking into consideration the direct toxicity of DON (and that of other mycotoxins) and its predisposing role in Clostridium perfringens-induced necrotic enteritis in chickens.

Pigs
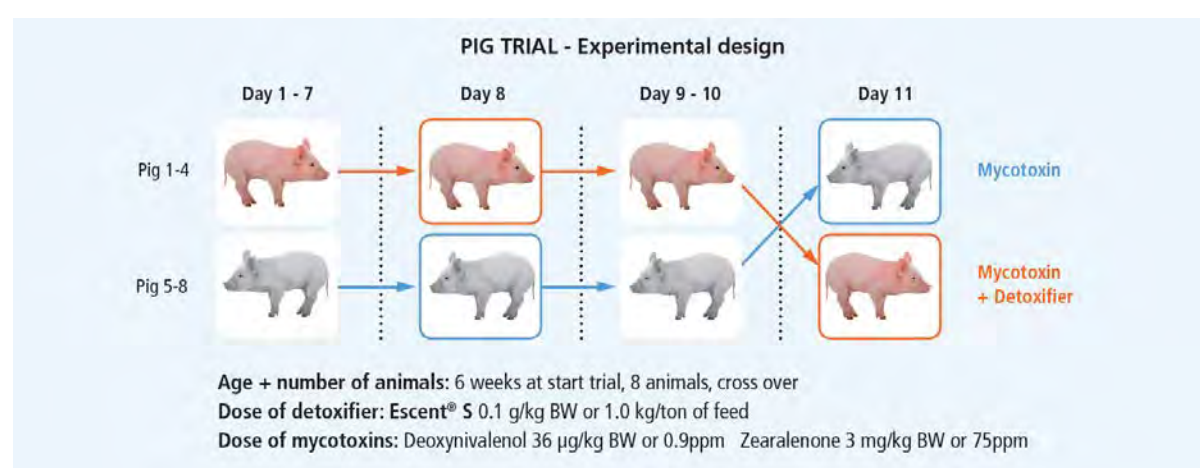
Fig. 3 Study design of the trials in pigs indicating number of animals per treatment, dose of detoxifier and dose of multiple mycotoxins.

Pigs treated with the detoxifier technology Escent® S exhibited a numerically reduced systemic concentration of DON and ZEN in plasma and urine and an increased concentration in faeces (Table 1) (Lauwers, Croubels, Letor, et al. 2019). In other words, Escent® S demonstrated a positive effect on pig detoxification despite the multi-mycotoxin contamination, as it succeeded in lowering the systemic exposure to DON and ZEN and it increased the amount of ZEN that was excreted via faeces. It should be noted that due to human error during the experiment, Escent® S was not applied at the recommended dose. Namely, Escent® S was applied only at 1kg/ton instead of the recommended 3kg/ton.

Table 1 Percentage (%) difference of the mean toxicokinetic area under curve determined after single oral administration of DON (0.9 ppm) and ZEN (75 ppm) to pigs, either with Escent® S (n=8) or without (Control, n= 8).

Biomarker of exposure	Treatment or Control	% difference of the toxicokinetic area under curve between Escent® S and Control
DON-GlcA in plasma	Escent® S	-13%
DON-GlcA in plasma	Control	
DON in urine	Escent® S	-26%
DON in urine	Control	
ZEN-GlcA in plasma	Escent® S	-12%
ZEN-GlcA in plasma	Control	
ZEN-GlcA in urine	Escent® S	-4%
ZEN-GlcA in urine	Control	
ZEN in faeces	Escent® S	+21%
ZEN in faeces	Control	

The findings are of particular importance taking into consideration the direct toxicity of DON and ZEN in pigs, their predisposing role in a spectrum of pig diseases and their negative impact on live PRSS vaccines.

The development of a novel Diagnostic Tool: Myco-marker®

The analytical methodology developed and validated for the detection of biomarkers of exposure in blood of chickens and pigs was then successfully transferred onto DBS with the application of mini volumes of blood on Whatman® 903 Protein Saver FTA paper cards. Table 2 shows a snapshot of the validation results for linearity across three different days of analysis of 23 mycotoxins of pig whole blood extracted from an 8 mm disk of dried blood spots (Lauwers, Croubels, De Baere, et al. 2019).

Myco-Marker® has now been translated into a new service (patent pending) to the industry for the evaluation of the overall risk of mycotoxins. Myco-Marker® is a diagnostic tool that combines the analytical detection of mycotoxins as well as phase I and phase II metabolites both in feed and in animal (chickens and pigs) blood. The DBS system offers great advantages to the end user including easy sampling as only a drop of blood is required (~60μl), reduced animal invasiveness, easier sample transportation without border restrictions and without any specific storage conditions, no need for plasma separation and no need for special procedures or tubes. The mycotoxin exposure data in animal blood allow, for the first time, the quantification with high accuracy the level of mycotoxins that enter and systemically expose the animal.

The Myco-Marker® diagnostic tool has the potential to evaluate not only the overall exposure of mycotoxins but also to assess their impact on animal health. It is only then when the producer can seek and assess appropriate mitigating strategies.

Table 2. Validation results for linearity shown as mean ± standard deviation of three curves across three different days of analysis (linear range, correlation coefficient (r) and goodness-of-fit coefficient (g)) and limit of quantification (LOQ) of 23 mycotoxins of pig whole blood extracted from an 8 mm disk of dried blood spots.

Analyte	LOQ (ng·mL^{-1})	Linearity (n=3 different days)	
		r ± SD	g ±SD
ZEN	1.0	0.994 ± 0.002	12.2 ± 3.6
AZEL	1.0	0.996 ± 0.002	13.2 ± 4.0
AZAL	1.0	0.996 ± 0.001	16.4 ± 3.0
BZAL	0.5	0.995 ± 0.003	18.8 ± 1.8
BZEL	0.5	0.995 ± 0.003	16.2 ± 3.7
ZAN	1.0	0.998 ± 0.002	14.3 ± 3.9
TeA	1.0	0.996 ± 0.002	19.3 ± 0.4
AOH	2.0	0.998 ± 0.001	18.1 ± 1.5
AME	1.0	0.997 ± 0.002	16.5 ± 3.6
DON	1.0	0.992 ± 0.002	14.8 ± 3.0
DOM1	1.0	0.995 ± 0.001	14.9 ± 2.2
3/15ADON	0.5	0.994 ± 0.003	17.7 ± 1.4
T2	0.5	0.998 ± 0.001	15.6 ± 4.9
AFB1	1.0	0.993 ± 0.002	11.8 ± 2.5
AFM1	0.5	0.996 ± 0.003	13.4 ± 1.62
OTA	1.0	0.996 ± 0.001	14.3 ± 4.9
ENNA1	0.5	0.995 ± 0.002	15.3 ± 3.1
ENNA	0.5	0.997 ± 0.002	17.5 ± 1.5
ENNB	0.5	0.997 ± 0.001	11.8 ± 6.8
ENNB1	1	0.995 ± 0.003	10.4 ± 1.9
BEA	0.5	0.993 ± 0.001	14.5 ± 3.3
FB1	1.0	0.995 ± 0.002	16.5 ± 2.6
FB2	1.0	0.998 ± 0.001	11.3 ± 5.1

Discussion

Mycotoxins in feed may impact greatly animal health and productivity. Feed analysis provides only a rough estimate of the risk involved in relation to the true amount of mycotoxins that animals could be potentially exposed to. Additionally, feed analysis is prone to significant methodological errors. The development of cost effective and reliable methods for determining the true exposure of mycotoxins to animals in field conditions has been the desire for several years.

The present evidence here relates to a) the evaluation of the efficacy of the detoxifier technology Escent® S with the use of biomarkers of exposure and b) the development of Myco-marker®, a novel diagnostic tool for enhancing productivity in the animal farming sector. In particular, Myco-marker® relates to a novel and inventive method for biomonitoring mycotoxins and their phase I and phase II metabolites, in an easy and user-friendly manner via accessible animal matrices, and more specifically in the blood of broiler chickens and pigs.

References:

Antonissen, G., F. Van Immerseel, F. Pasmans, R. Ducatelle, F. Haesebrouck, et al. 2014. The mycotoxin deoxynivalenol predisposes for the development of Clostridium perfringens-induced necrotic enteritis in broiler chickens. PLoS One. 2014;9(9): e108775. doi: 10.1371/journal.pone.0108775.

Antonissen G., S. Croubels, F. Pasmans, et al. 2015. Fumonisins affect the intestinal microbial homeostasis in broiler chickens, predisposing to necrotic enteritis. Vet Res. 2015; 46(1):98. doi:10.1186/s13567-015-0234-8.

Eskola M., G. Kos, CT. Elliot et al. 2019. Worldwide contamination of food-crops with mycotoxins: Validity of the widely cited 'FAO estimate' of 25%. Crit Rev in Food Sci and Nutr, DOI: 10.1080/10408398.2019.1658570

Kovalsky P., G. Kos, C. Schwab et al. 2016. Co-Occurrence of Regulated, Masked and Emerging Mycotoxins and Secondary Metabolites in Finished Feed and Maize-An Extensive Survey. Toxins; 6;8(12). pii: E363, DOI:10.3390/toxins8120363

Lauwers M., S. Croubels, S. De Baere, et al. 2019. Assessment of Dried Blood Spots for Multi-Mycotoxin Biomarker Analysis in Pigs and Broiler Chickens. Toxins;11(9):541. doi:10.3390/toxins11090541.

Lauwers M., S. Croubels, B. Letor, C. Gougoulias, M. Devreese. 2019. Biomarkers for Exposure as A Tool for Efficacy Testing of A Mycotoxin Detoxifier in Broiler Chickens and Pigs. Toxins;11(4):187. doi:10.3390/toxins11040187.

Savard C., CA. Gagnon, Y. Chorfi. 2015. Deoxynivalenol (DON) naturally contaminated feed impairs the immune response induced by porcine reproductive and respiratory syndrome virus (PRRSV) live attenuated vaccine. Vaccine; 33(32): 3881–3886. doi: 10.1016/j.vaccine.2015.06.069.

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Bacterial Debris – Peptidoglycans and Their Influence on Gut Functionality

Débris bactériens – les peptidoglycanes et leur influence sur la fonctionnalité intestinale

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Abstract

The gastrointestinal tract (GIT) is host to a rich and complex microbial ecosystem that has been associated with several host functions including intestinal development, nutrient absorption and energy metabolism.

A widespread used technique to study the microbiota is based on molecular DNA sequencing methods that use genome sequences to identify microbes. This approach has dramatically advanced our understanding of the microbiome. The methods however don't provide information about the physiological state of the GIT bacteria (are they active, inactive, or dead?) as well as abundance of bacterial waste products.

Bacterial waste products (debris) in the GIT can be quantified by analysis of microbial structural patterns specific to bacteria. One abundant microbial polymer unique to bacteria is the cell wall component peptidoglycan. Peptidoglycan makes up approximately 90% of gram-positive bacteria cell walls and is also a minor component in gram-negative bacterial cell walls. Recent published research has reported a beneficial effect of enzymatic peptidoglycan hydrolysis in the GIT of chickens, thus suggesting that peptidoglycan influence gastrointestinal functionality.

To better understand the peptidoglycan - GIT interplay better, we established a mass spectroscopy-based method to quantify soluble and total peptidoglycan concentrations in intestinal content

samples by measuring the peptidoglycan building block muramic acid. The method proved capable of quantifying soluble and total peptidoglycan concentrations in digesta samples from chickens. The method was also sensitive enough to measure increased concentrations of soluble peptidoglycan when a peptidoglycan hydrolyzing enzyme was supplemented to the diet.

Résumé

Le tractus gastrointestinal (TGI) est l'hôte d'un écosystème microbien riche et complexe qui a été associé à plusieurs fonctions, notamment le développement intestinal, l'absorption des nutriments et le métabolisme énergétique.

Le séquençage de l'ADN moléculaire reposant sur les séquences du génome permettant d'identifier les microbes est une technique largement utilisée pour étudier le microbiote. Cette approche a fait progresser de façon spectaculaire notre compréhension du microbiome. Les méthodes ne fournissent cependant aucune information sur l'état physiologique des bactéries du TGI (sont-elles actives, inactives ou mortes?) ni sur l'abondance des déchets bactériens.

Les déchets bactériens (débris) dans le TGI peuvent être quantifiés par l'analyse des modèles structurels microbiens spécifiques aux bactéries. Le peptidoglycane, un composant de la paroi cellulaire, est un polymère microbien abondant et unique aux bactéries. Le peptidoglycane constitue environ 90 % des parois cellulaires des bactéries à Gram positif et est également un composant mineur des parois cellulaires des bactéries à Gram négatif. Des travaux publiés récemment ont rapporté un effet bénéfique de l'hydrolyse enzymatique du peptidoglycane dans le TGI des poulets, suggérant ainsi que le peptidoglycane influence la fonctionnalité gastrointestinale.

Pour mieux comprendre l'interaction peptidoglycane - TGI, nous avons établi une méthode basée sur la spectroscopie de masse pour déterminer les concentrations de peptidoglycanes solubles et totaux dans les échantillons de contenu intestinal par la mesure de l'acide muramique, élément constitutif du peptidoglycane. La méthode a permis de quantifier les concentrations de peptidoglycanes solubles et totaux dans des échantillons de digesta de poulets. La méthode s'est aussi montrée suffisamment sensible pour mesurer l'augmentation des concentrations de peptidoglycanes solubles après l'ajout dans la ration d'une enzyme hydrolysant le peptidoglycane.

Introduction

Although bacteria are too small to be seen without the aid of a microscope, their abundance by mass has been estimated to be 1,166 times larger than the mass of all humans. All animals contain populations of bacteria on outer and inner body surfaces such as the skin and the gastrointestinal tract, with the gastrointestinal tract being by far the most densely populated. It has been estimated that cattle, sheep, goats, pigs, chickens, ducks and turkey contain between 2.1×10^{10} and 3.2×10^{11} bacteria per gram of intestinal wet contents (Bar-On, Phillips and Milo, 2018; Whitman, Coleman and Wiebe, 1998)

The bacterial ecosystem in the gastrointestinal tract closely interact with the host and has received tremendous attention by researchers in recent decades. Despite all the mechanisms discovered to date the field remains at the beginning of an era with respect to solutions that can manipulate the ecosystem to the benefit of gastrointestinal functionality. One number illustrating the complexity of the bacterial gastrointestinal community is that it contains an estimated 150 times more unique genes than its host (Cani *et al.*, 2019).

In an early report of bacterial gut ecosystem manipulation, Italian anatomist and surgeon Acquapendente (1537-1619) coined "transfaunation" when he transferred gastrointestinal contents from healthy to sick animals to improve gastrointestinal functionality. Many animals practice coprophagia, leading to transfer of beneficial and pathogenic bacterial populations between animals (de Groot *et al.*, 2017).

The most widely used research tools to describe changes in the bacterial ecosystem (which bacteria are present) and metabolic potential (which genes are present) are based on sequencing the phylogenetic marker gene 16S ribosomal RNA or genome DNA. These tools are extremely powerful but fail to answer basic questions about physiological status or if the bacteria are dead or alive (Maurice, Haiser and Turnbaugh, 2013; Ben-Amor *et al.*, 2005). Another limitation of the widely applied sequencing tools is that they cannot quantitate the components of bacterial cell debris in the gut. The differentiation between live cells, injured cells, dead cell and cell debris is important because each unity will interact with the host differently.

Live cells produce metabolites that can interact with the host, two examples being short chain fatty acids and branched chain amino acids. Another way live cells interact with metabolism is through the release of bacterial cell structure components during cell division that can affect host metabolism by interacting with the immune system. Here, two examples are lipopolysaccharides (endotoxin) and peptidoglycan. Upon bacterial death, molecules are released that otherwise are hidden in the cytoplasm or cell wall of the living cell. Many of these molecules are unique to bacteria and recognized by the innate immune system. Well known examples are LPS, peptidoglycan, teichoic acid and unmethylated CpG DNA motifs (Stewart-Tull, 1980; Akira, Uematsu and Takeuchi, 2006). The impact of dead bacterial components on host response have been demonstrated for both gram-negative and gram-positive bacteria through changes in body temperature as a response to intravenous infusion in rabbits. In one example with gram-positive bacteria (that does not have the potent pyrogen LPS in the cell wall), fever developed to a similar level for either dead or live bacteria (Kluger and Matthew, 2015).

Abundance of live and dead bacteria in the gastrointestinal tract

Only six studies have reported the relative abundance of live and dead cells in the gastrointestinal tract (Figure 1). Two human studies have looked at fecal sample live/dead/injured cell ratio by fluorescence activated cell sorting. The first study from 2005 found 49% intact cells 19% damaged cell and 32 % dead cells. The second study found 56% intact cells, 27% damaged cells and 17% dead cells, and observed a wide range of variation between individuals. Dead bacterial % was in the range of 6% - 41% (Maurice, Haiser and Turnbaugh, 2013; Ben-Amor *et al.*, 2005). Cecal samples from Syrian hamsters and arctic ground squirrels contained 72% - 81% intact cells, 4% -

9% damaged cells, and 10% - 20% dead cells ((Hatton *et al.*, 2017; Stevenson, Duddleston and Buck, 2014; Sonoyama *et al.*, 2009). Yet another study used a live/dead PCR approach to measure live and dead cells in Rex rabbits, and noted that 1% - 3% live cells were found in the foregut (stomach, jejunum, ileum), 25% in the cecum and 19% in the colon. Injured cells are not quantified by live/dead PCR (Fu *et al.*, 2018).
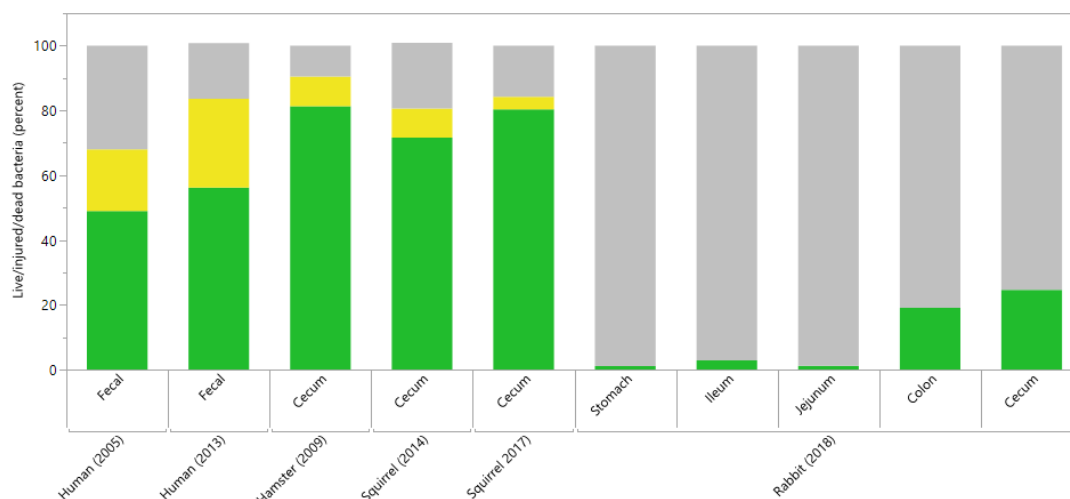
Figure 1 Literature estimates of percent live, injured and dead cell in intestinal samples from human, hamster, squirrel and rabbit. Green bars show % live cells, yellow bars show percent injured cells and grey cells show % dead cell as estimated by flow cytometry (human/hamster/squirrel) and live/dead PCR (rabbit)

DNA independent methods are needed to quantify amounts of debris from cells that are decomposed to a level where DNA is no longer is contained within the cell wall. One major component of bacterial cell debris is the key structural cell wall polymer peptidoglycan. Peptidoglycan's role for the bacterial cell is to protect cell integrity against tugor pressure, and build cell shape as well as being an anchor point for other cell envelope components such as proteins and teichoic acid (Vollmer, 2008). As peptidoglycan and some of its buildings blocks are uniquely found in the bacterial cell walls, it also functions as a host immune system recognition target (Stewart-Tull, 1980). The uniqueness of peptidoglycan and, more specifically, the sugar component muramic acid – has made it a biomarker target to estimate bacterial abundance in soil, as well as in cattle feces (Joergensen, 2018; Jost *et al.*, 2013).

Quantification of soluble and total peptidoglycan in the gastrointestinal tract

Literature reports on the physiological effects of enzymatic hydrolysis of peptidoglycan from cell debris in the gastrointestinal tract of chickens prompted us to determine if muramic acid could be used as an indicator of peptidoglycan hydrolysis (Lichtenberg *et al.*, 2017; Goodarzi Boroojeni *et al.*, 2019; Sais *et al.*, 2019). Peptidoglycan is the only source of muramic acid in the intestinal tract.

Hydrolyzed peptidoglycan *in vitro* is more soluble than intact peptidoglycan. The principle of measuring enzyme hydrolysis by quantifying substrate solubilization in digesta samples has successfully measured the effect of carbohydrases (Choct *et al.*, 2004; Rosenthal and Dziarski, 1994).

Briefly, total muramic acid was determined in freeze dried digesta samples similarly as in soil science (Joergensen, 2018). In addition, soluble muramic acid was measured in the supernatant from a slurry of freeze dried digesta from the sample used for determination of total muramic acid. The ratio of soluble peptidoglycan as a % of total was used as a measure of peptidoglycan hydrolysis.

Analysis of chicken intestinal samples from the crop, jejunum and caecum confirmed that a supplemented muramidase (dose 45,000 LSU(F)/kg) increased the relative abundance of soluble peptidoglycan compared to intact peptidoglycan (Figure 2). In crop, soluble peptidoglycan as a % of total increased from 37% (n=12) in control group to 46% (n=9) in muramidase supplemented group (n=9). In jejunum, soluble peptidoglycan as a % of total increased from 32% (n=11) in the control group to 66% (n=11) in the muramidase supplemented group. In cecum, soluble peptidoglycan as a % of total increased from 9% (n=10) in control group to 16% (n=12) in muramidase supplemented group.
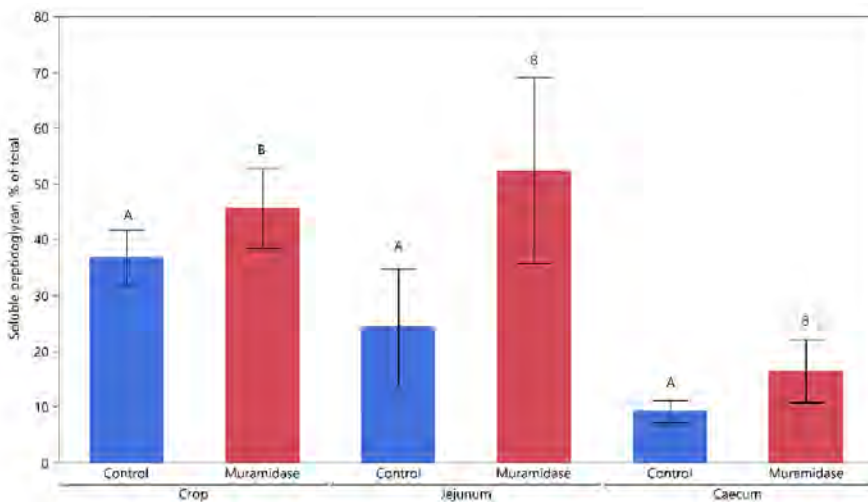
Figure 2 Soluble peptidoglycan, % of total, in chicken crop, jejunum and caecum intestinal samples. Muramidase supplemented diets have significant higher soluble peptidoglycan, % of total. Bars with different letters A and B are significant different, within each intestinal segment in a student's t-test (p = 0.05).

A first step towards understanding the role of the bacterial macromolecule peptidoglycan in the gastrointestinal tract is to develop analytical methods for its quantification. Here, we find that muramic acid in the digesta can estimate the ratio between soluble and total peptidoglycan in the

gut. This provides new knowledge about the interaction of peptidoglycan hydrolysis with gastrointestinal functionality and animal physiology.

References

Akira, S., Uematsu, S. and Takeuchi, O. (2006) 'Pathogen recognition and innate immunity', *Cell,* 124(4), pp. 783-801.

Bar-On, Y. M., Phillips, R. and Milo, R. (2018) 'The biomass distribution on Earth', *Proc Natl Acad Sci U S A,* 115(25), pp. 6506-6511.

Ben-Amor, K., Heilig, H., Smidt, H., Vaughan, E. E., Abee, T. and de Vos, W. M. (2005) 'Genetic diversity of viable, injured, and dead fecal bacteria assessed by fluorescence-activated cell sorting and 16S rRNA gene analysis', *Appl Environ Microbiol,* 71(8), pp. 4679-89.

Cani, P. D., Van Hul, M., Lefort, C., Depommier, C., Rastelli, M. and Everard, A. (2019) 'Microbial regulation of organismal energy homeostasis', *Nature Metabolism,* 1(1), pp. 34-46.

Choct, M., Kocher, A., Waters, D. L., Pettersson, D. and Ross, G. (2004) 'A comparison of three xylanases on the nutritive value of two wheats for broiler chickens', *Br J Nutr,* 92(1), pp. 53-61.

de Groot, P. F., Frissen, M. N., de Clercq, N. C. and Nieuwdorp, M. (2017) 'Fecal microbiota transplantation in metabolic syndrome: History, present and future', *Gut Microbes,* 8(3), pp. 253-267.

Fu, X., Zeng, B., Wang, P., Wang, L., Wen, B., Li, Y., Liu, H., Bai, S. and Jia, G. (2018) 'Microbiome of Total Versus Live Bacteria in the Gut of Rex Rabbits', *Front Microbiol,* 9, pp. 733.

Goodarzi Boroojeni, F., Männer, K., Rieger, J., Pérez Calvo, E. and Zentek, J. (2019) 'Evaluation of a microbial muramidase supplementation on growth performance, apparent ileal digestibility, and intestinal histology of broiler chickens', *Poult Sci,* 98(5), pp. 2080-2086.

Hatton, J. J., Stevenson, T. J., Buck, C. L. and Duddleston, K. N. (2017) 'Diet affects arctic ground squirrel gut microbial metatranscriptome independent of community structure', *Environ Microbiol,* 19(4), pp. 1518-1535.

Joergensen, R. G. (2018) 'Amino sugars as specific indices for fungal and bacterial residues in soil', *Biology and Fertility of Soils,* 54(5), pp. 559-568.

Jost, D. I., Aschemann, M., Lebzien, P., Joergensen, R. G. and Sundrum, A. (2013) 'Microbial biomass in faeces of dairy cows affected by a nitrogen deficient diet', *Arch Anim Nutr,* 67(2), pp. 104-18.

Kluger and Matthew (2015) *Fever: Its Biology, Evolution, and Function. Princeton Legacy Library*: Princeton University Press.

Lichtenberg, J., Perez Calvo, E., Madsen, K., Østergaard Lund, T., Kramer Birkved, F., van Cauwenberghe, S., Mourier, M., Wulf-Andersen, L., Jansman, A. J. M. and Lopez-Ulibarri, R. (2017) 'Safety evaluation of a novel muramidase for feed application', *Regul Toxicol Pharmacol,* 89, pp. 57-69.

Maurice, C. F., Haiser, H. J. and Turnbaugh, P. J. (2013) 'Xenobiotics shape the physiology and gene expression of the active human gut microbiome', *Cell,* 152(1-2), pp. 39-50.

Rosenthal, R. S. and Dziarski, R. (1994) 'Isolation of peptidoglycan and soluble peptidoglycan fragments', *Methods Enzymol,* 235, pp. 253-85.

Sais, M., Barroeta, A. C., López-Colom, P., Nofrarías, M., Majó, N., Lopez-Ulibarri, R., Pérez Calvo, E. and Martín-Orúe, S. M. (2019) 'Evaluation of dietary supplementation of a novel microbial muramidase on gastrointestinal functionality and growth performance in broiler chickens', *Poult Sci.*

Sonoyama, K., Fujiwara, R., Takemura, N., Ogasawara, T., Watanabe, J., Ito, H. and Morita, T. (2009) 'Response of gut microbiota to fasting and hibernation in Syrian hamsters', *Appl Environ Microbiol,* 75(20), pp. 6451-6.

Stevenson, T. J., Duddleston, K. N. and Buck, C. L. (2014) 'Effects of season and host physiological state on the diversity, density, and activity of the arctic ground squirrel cecal microbiota', *Appl Environ Microbiol,* 80(18), pp. 5611-22.

Stewart-Tull, D. E. (1980) 'The immunological activities of bacterial peptidoglycans', *Annu Rev Microbiol,* 34, pp. 311-40.

Vollmer, W. (2008) 'Structural variation in the glycan strands of bacterial peptidoglycan', *FEMS Microbiol Rev,* 32(2), pp. 287-306.

Whitman, W. B., Coleman, D. C. and Wiebe, W. J. (1998) 'Prokaryotes: the unseen majority', *Proc Natl Acad Sci U S A,* 95(12), pp. 6578-83.
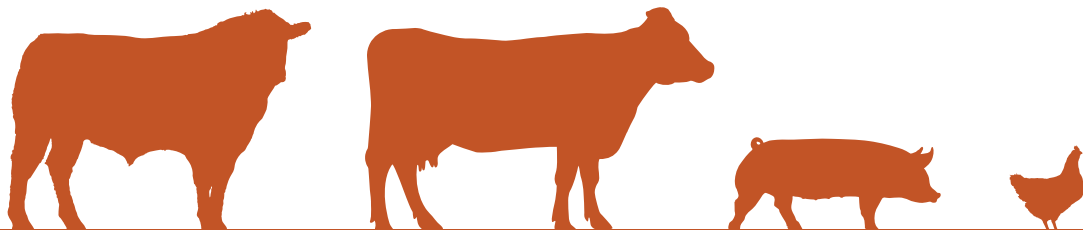
The impact of trace mineral form and source on premix and feed component stability

L'impact de la forme et de la source des oligo-éléments sur la stabilité des composants des prémélanges et des aliments

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Abstract

Organic trace minerals (OTMs) are recognised globally as being a more bioavailable source of mineral than their inorganic counterparts. Whilst there are many forms of mineral products available in the marketplace for use in animal nutrition; these have unfortunately been generically entitled 'organic trace minerals' by virtue of the fact that the trace elements in question are complexed or otherwise associated with organic molecules.

Typically speaking, OTMs can be produced through numerous mechanisms depending on the trace mineral product being manufactured. The process of complexing or chelating elements such as copper, iron or zinc for instance typically involves reacting inorganic mineral salts with a suitable bonding group such as a peptide or amino acid, after which the mineral becomes part of a biologically stable structure. Numerous production processes have been developed, ranging from highly specific and controlled reaction processes to more involved chemical synthesis routes.

Given the vastly different products which exist in the marketplace, the importance of understanding the physical differences between them cannot be understated. A greater understanding of the basics behind these products will allow end-users to differentiate between them not only in terms of their physical make-up but also in terms of likely behaviour *in-vivo*.

Résumé

Les oligo-éléments organiques sont reconnus dans le monde entier comme étant une source de minéraux plus biodisponible que leurs homologues inorganiques. Bien qu'il existe de nombreuses formes de produits minéraux offertes sur le marché pour l'alimentation animale, celles-ci ont malheureusement été regroupées sous l'appellation générique d' « oligo-éléments organiques » en raison du fait que les micronutriments en question sont liés sous la forme de complexes ou sont autrement associés à des molécules organiques.

Règle générale, les oligo-éléments organiques peuvent être produits par de nombreux mécanismes, selon le micronutriment fabriqué. Le processus de complexation ou de chélation d'éléments tels que le cuivre, le fer ou le zinc, par exemple, implique généralement la réaction de sels minéraux inorganiques avec un groupe de liaison approprié tel qu'un peptide ou un acide aminé, après quoi le minéral devient partie intégrante d'une structure biologiquement stable. De nombreux procédés de production ont été mis au point, allant de processus de réaction hautement spécifiques et contrôlés à des voies de synthèse chimique plus complexes. Compte tenu de la grande diversité des produits qui existent sur le marché, on ne saurait sous-estimer l'importance de comprendre les différences physiques qui existent entre eux. Une meilleure connaissance des principes de base de ces produits permettra aux utilisateurs finaux de les différencier non seulement en termes de constitution physique, mais aussi en termes de comportement probable *in vivo*.

Complexes or chelates?

Generically speaking, the term 'complex' can be used to describe the product formed when a metal ion reacts with a bonding group or ligand that contains an atom which has a lone pair of electrons. In the complex, the ligand is bonded to the metal ion through donor atoms such as oxygen, nitrogen or sulphur. Ligands that contain only one donor atom are termed 'monodentate', whilst those that contain two or more donor atoms capable of bonding to a metal ion are termed bi-, tri or tetradentate. These multi donor species can also be referred to as polydentate (Byrne, 2010)

When such ligands bond to a metal ion via two or more donor atoms on the same ligand, the compIex formed contains one or more heterocyclic rings which contain the metal atom. Such complexes are termed as 'chelates' (from the Greek; *chele,* referring to a crab's claw).

Amino acids such as glycine are examples of bidentate Iigands which bond to metal ions via an oxygen of the carboxylic acid group and the nitrogen of the amino group. In contrast, ethylenediaminetetraacetic acid (EDTA) is an example of a hexadentate ligand containing six donor atoms. It forms highly stable complexes with most metal ions and in fact is not particularly useful for the formation of mineral chelates as the bioavailability of such complexes is negligible (Byrne, 2010).

It must also be remembered that while all chelates are complexes, *not all complexes are chelates*. Indeed, whilst the overall theory behind chelation is simple, there are a number of criteria which must be absolutely met in order to ensure the generation of a *stable mineral chelate*

- A chelating ligand must contain at least two atoms capable of forming bonds with the metal ion
- The ligand must form a heterocyclic ring with the metal as the closing member of the ring
- It must be physically (sterically) possible to chelate the metal
- The ratio of the ligand to the mineral must meet minimum requirements for stability

True chelates have the 'ring structure' formed by bonding between the amino and carboxyl ends of the amino acid and the metal ion.

Amino acids and peptides as ligands

The chemistry behind OTMs has created a great deal of confusion in the animal feed industry. Terms such as metal amino acid complexes, metal amino acid chelates, metal polysaccharide complexes and metal proteinates abound, yet official definitions remain vague and unhelpful. As an example, definitions of the most common organic trace minerals used in agricultural practice as laid down by the Association of American Feed Control Officials (AAFCO, 1998) are illustrated in table 1.

Table 1. AAFCO definitions for organic mineral complexes (AAFCO, 1998).

Metal Amino Acid Complex – is the product resulting from complexing of a soluble metal salt with an amino acid(s).
Metal Amino Acid Chelate – is the product resulting from the reaction of a metal ion from a soluble metal salt with amino acids with a mole ratio of one mole of metal to one to three (preferably two) moles of amino acids to form coordinate covalent bonds. The average weight of the hydrolyzed amino acids must be approximately 150 and the resulting molecular weight of the chelate must not exceed 800.
Metal Polysaccharide Complex – is the product resulting from complexing of a soluble salt with a polysaccharide solution.
Metal Proteinate – is the product resulting from the chelation of a soluble salt with amino acids and / or partially hydrolyzed protein.

In deference to the AAFCO definitions, table 2 gives an overview of the EU classification of organic zinc products from which one can appreciate the stark differences between the official terminologies used for regulatory control and the obvious confusion that can occur when comparing products.

Table 2. EU classification of organic zinc products

Zinc chelate of amino acids hydrate - Zinc amino acid complex where the zinc and the amino acids derived from soya protein are chelated via coordinate covalent bonds, as a powder with a minimum content of 10 % zinc.   Characterisation of the active substance: Chemical formula: $Zn(x)1–3nH_2O$, x = anion of any amino acid from soya protein hydrolysate. Maximum of 10 % of the molecules exceeding 1 500 Da.
Zinc chelate of protein hydrolysates - Zinc chelate of protein hydrolysates as a powder with a minimum content of 10 % zinc. Minimum of 85 % zinc chelated.   Characterisation of the active substance Chemical formula: $Zn(x)1–3 \cdot nH_2O$, x = anion of protein hydrolysates containing any amino acid from soya protein hydrolysate
Zinc chelate of glycine hydrate (solid) - Zinc chelate of glycine, hydrate, as a powder with a minimum content of 15 % zinc. Moisture: maximum 10 %.   Characterisation of the active substance Chemical formula: $Zn(x)1-3 \cdot nH_2O$, x = anion of glycine
Zinc chelate of glycine hydrate (liquid) - Liquid zinc chelate of glycine, hydrate, with a minimum content of 7 % zinc.   Characterisation of the active substance Chemical formula: $Zn(x)1-3 \cdot nH_2O$, x = anion of glycine.

An important feature of organic chelates is their enhanced stability due to the conformation in which the metal is held by the bonding group(s). In general terms, the stability of a chelate is the defining characteristic of its bioavailability and chelates with low stabilities will see the effectiveness of the product reduced to that of the corresponding inorganic salt. Individual bonding groups such as amino acids or peptides, have the potential to form complexes or chelates with varying degrees of stability. It is reasonable to expect that bonding groups, such as peptides, which have a greater number of donor atoms would have higher stabilities than simple amino acids such as glycine. Arising from this, one would anticipate that peptide based chelates will have the necessary physico-chemical properties to ensure wide ranging stability under conditions of changing pH (Murphy, 2018a, 2018b).

Stabilising a chelate – the effect of ligand.

When trying to compare chelates or complexes on the basis of "which is best under this set of conditions", one really needs to consider many different factors. However, it is useful to compare products in terms of what's known as their stability constant. A stability constant (also known as a formation constant or a binding constant) is an equilibrium value for the formation of a complex or chelate in solution (Martell and Motekaitis, 1992). The overall stability constant is the product of all stepwise stability constants. For example, if K1 and K2 are the stability constants for the addition of the first and second ligand respectively, then the overall stability constant (β_2) is K1 x K2.

This value is a relative measure of the strength of the interaction between a metal and the ligand in a chelate or complex.

We can derive this value by measuring the relative proportions of metal ([M]), ligand ([L]) and chelate ([ML])

$$[L] + [M] \leftrightarrow [ML]$$
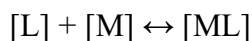
Note that there are a number of influencing factors which play a role in this equilibrium:
- pH significantly influences the equilibrium between ML and L+M.
- Additional factors also influence this, such as the type and makeup of ligand, relative proportions of [L] to [M] etc.

Ultimately the stability constant, β, can be defined as a measure of the ratio of the chelate concentration to the concentrations of the free metal and ligand under a given set of conditions (Martell and Motekaitis, 1992). For simplicity it can be represented as follows:

$$\beta = [ML] / [L][M]$$

Essentially, this tells us is that the greater the value of the stability constant β, the greater the proportion of the chelate or complex which is present relative to free ligand ([L]) or free metal ([M]) at a given pH. Typically, the stability constant is presented in log values and can serve as a useful guide when comparing different bonding groups.

The stability constants for a range of ligands including single amino acids, di-peptides, tri-peptides etc can be readily obtained from the NIST stability constants database which calculates the value considering relative pH, ionic strength, temperature, ligand type, ligand and metal concentrations (NIST, 2004).

Consider the data in table 3 which compares a range of ligands when complexed with copper under the same physiological conditions. For simplicity, the stability values (log data) have been transformed and compared on a relative basis to that of glycine. The molecular weight of each ligand is also indicated. As the type of bonding group changes, so too does the strength of bond between it and the copper group - some bond copper tightly and some bond copper very weakly. The same holds true for all other minerals such as Zn, Mn and Fe.

Table 3. Relative stabilities of organically bound copper complexes.

Bonding group	Relative Stability
Propionic acid (74Da)	1×10^{-6}
Methionine Hydroxy Analogue (150Da)	2.63×10^{-6}
Met (m.wt. 149Da)	0.5
Gly (m.wt. 75Da)	1
His-Ser (m.wt. 260Da)	2.5
His-Met (m.wt. 304Da)	2.5
Gly-Cys (m.wt. 196Da)	21
Gly-Lys (m.wt. 221 Da)	2818
Tyr-Trp (m.wt. 385Da)	3235
Ala-Lys (m.wt. 238Da)	9549
Tyr-Lys (m.wt. 327Da)	186208
EDTA	5.6×10^{10}

Adapted from: Critically selected stability constants of metal complexes, NIST Database 46 (NIST, 2004)

What this indicates is that the size of the bonding group is not the most critical factor influencing bond strength and ultimately stability of a chelate. Claims of superiority based on size clearly have little merit. However, simply increasing the number of amino acids in a ligand may not increase the stability of the metal complex and thus may not necessarily increase the relative proportion of bound mineral (Byrne, 2010; Byrne *et al*, 2011).

Ultimately, not only does the type of amino acid influence the stability of a given chelate but the position of amino acids in a peptide can also significantly influence how the ligand and mineral interact. This is illustrated in table 4 where it can be appreciated that the most critical factors are the sequence and position of amino acids rather than the overall size.

Table 4. Role of amino acid sequence on chelate bond strength and stability.

Bonding Group	Relative stability
Gly-Gly-Gly (m.wt 225Da)	1
Gly-Gly-His (m.wt. 305Da)	270
Gly-His-Gly (m.wt. 305Da)	8511

The substitution of a histidine into the tripeptide Gly-Gly-Gly to yield Gly-Gly-His for instance enhances the stability value and thus the relative proportion of bound mineral (copper in this instance). Furthermore, changing the position of this histidine within the tripeptide sequence (to form Gly-His-Gly for example) can result in a further increase in the bond strength and as such an increase in the proportion of bound mineral (Byrne *et al*, 2018).

In practical terms, simple changes in the configuration of amino acids in this tripeptide result in a greater proportion of bound mineral relative to free mineral and ligand. Essentially, mineral chelate stability can be significantly influenced by not only the type of amino acid but also the configuration of amino acids in a peptide sequence.

From a production standpoint, it is important to note that the extent and type of hydrolysis of a protein source to form short chain peptides can significantly influence the sequence of amino acids present in these peptides. The production of an 'optimal' protein hydrolysate for mineral chelation can be effected through careful selection of the hydrolysis conditions. This ensures that the peptide hydrolysate will have the necessary properties to ensure constancy and mineral binding stability under conditions of changing pH.

A recent study using potentiometric-based techniques analysed a range of commercial OTMs using a Cu ion-selective electrode to determine their *in vitro* stabilities over a pH range reflective of physiological conditions (Figure 1). In this work, samples were reconstituted and suspended prior to titration of the supernatants, with subsequent measurement of the percentage bound copper over a pH range of 3 to 8 (Byrne *et al*, 2018). This confirmed that notable differences exist in the pH-dependant stability of commercial OTMs, with the amount of bound copper varying considerably between samples. Furthermore, the data indicates that some OTMs have low or no capacity for stable mineral bonding at acidic pH, with obvious impacts on the bioefficacy of the products. These differences can be attributed to not only the type of bonding group used but also to the production process used to generate same.
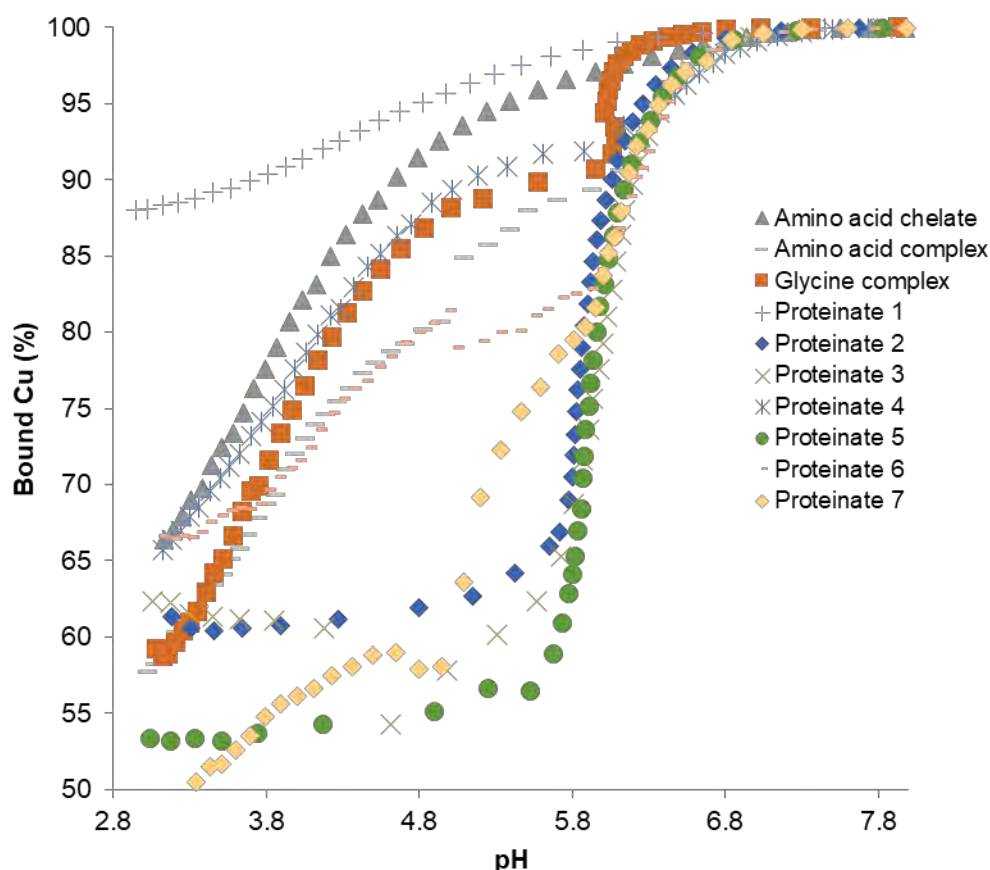
Figure 1. Potentiometric titrations of commercial copper (II) chelates

Ultimately, the stability of an OTM is of paramount importance to its bioavailability. During transit through the GI tract and as the pH decreases or acidifies, all OTMs are subjected to physiological forces which can result in the bound mineral complex dissociating and releasing free mineral ions. There are a number of negative consequences to this pH induced dissociation of OTMs (Murphy, 2017). For instance, the charged free mineral ion can react with negatively charged plant components such as phytic acid which may be present in the GI tract or worse still can form so-called hydroxides upon reaching the more alkaline environment in the intestine. This can lead to the phenomenon of pH induced hydroxypolymerisation and result in precipitation of the mineral and thus lead to a very significant reduction in bioavailability.

Essentially, complexes or chelates with low stabilities will not deliver the mineral to the sites of absorption in the intestine and reduce the effectiveness of the product to that of the corresponding inorganic salt. We need to maximise the pH dependant stability of OTMs to increase mineral uptake in the intestine. In essence, the higher the stability of an OTM, the greater it's bioavailability is likely to be.

Premix and feed antagonisms

Increasingly, the agonistic and antagonistic effects of feed components have come under scrutiny, with choice of components gaining increasing importance in diet formulation. The

possibility for negative interactions occurring between individual components within premixes and feeds is high and often overlooked as are the underlying effects at a cellular level following digestion and absorption of the mineral source (Murphy, 2016a)

Recent studies have focused on assessing these potential antagonisms. The differential effects noted indicates that not all chelates are created equal; moreover, they all differ in terms of their stabilities, releasing mineral in a pH dependent fashion based on the pH in the local micro-environment. This instability results in some chelates having a negative impact on premix and feed components (Murphy, 2018b).

Data detailing the potential *in vitro* interaction between inorganic and organic chelated sources of Fe, Zn and Cu with three commercially available phytase preparations illustrates the potential for component based antagonism (O'Rourke *et al*, 2016; Santos *et al*, 2015). The study also investigated if the degree of enzyme inhibition was dependent of the type of OTM used as mineral source. The authors demonstrated that a highly significant relationship between phytase inhibition, trace mineral type as well as mineral source and concentration existed. Peptide based chelates were consistently and significantly less inhibitory than the majority of the other sources (Table 5).

The consequences that this mineral induced inhibition of enzyme activity has for premix and feed formulation are tremendous and go some way towards explaining the variation noted in supplementation response. It may well be that the trend towards super-dosing of phytase activities in diets is an unintentional consequence of the negative interactions of premix components.

Table 5. IC$_{50}$ values (ppm) for inhibition of phytase by iron

	IC$_{50}$ Concentration (ppm)				
	PRO	**GLY**	**ACH**	**PSC**	**SO$_4$**
E. coli	6.7 ± 1.1 [A]	3.4 ± 0.3 [B C]	0.9 ± 0.1 [C]	3.7 ± 0.1 [B]	4.1 ± 0.4 [B]
P. lycii	16.7 ± 2.5 [A]	3.8 ± 0.8 [B]	1.4 ± 0.2 [B]	10.4 ± 1.3 [C]	2.9 ± 0.4 [B]
A. niger	10.9 ± 1.0 [A]	6.0 ± 0.9 [B]	1.1 ± 0.1 [C]	12.0 ± 1.3 [A]	8.5 ± 1.0 [A B]

IC$_{50}$ values are the means ± SD (*n = 2*) that represent the upper and lower limits of the interpolated dose-inhibition curves with 95 % confidence
For each IC$_{50}$, source means marked by different letters significantly differ (*p<0.05*; Tukey's HSD post-hoc tests)
PRO, proteinate; GLY, glycinate; ACH, amino acid chelate; PSC, polysaccharide complex; SO$_4$, sulphate

Additional research assessing the effect of chelate source and concentration on the efficacy of antioxidants such as BHT has established that commonly used antioxidants can be compromised by the use of inorganic minerals (Figure 2). The data further indicates that in some cases, chelates can also have a significant negative effect on antioxidant activity. Essentially, the use of weakly chelated minerals may result in a reduction in the efficacy of feed antioxidants such as BHT. The use of chelated minerals with high stability may result in a sparing effect on antioxidant activity (Concarr *et al*, 2016).
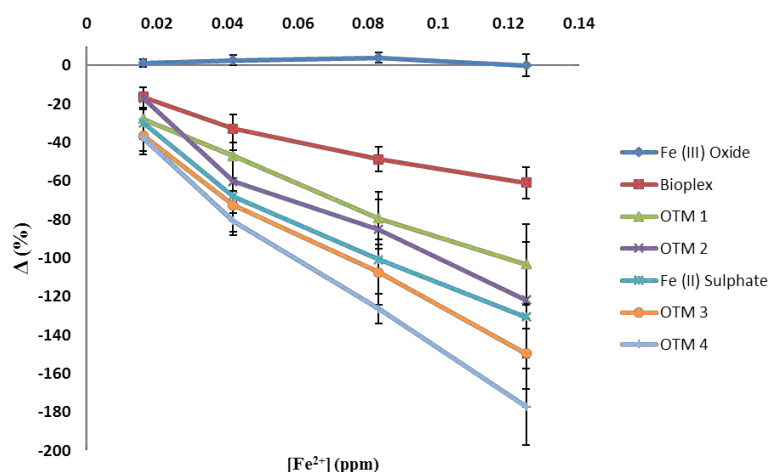
Figure 2. Relative activity of antioxidant (BHT) following exposure to organic (OTM) and Inorganic (ITM) sources of Fe^{2+}

In trace mineral premixes, oxidation-reduction reactions are the predominant cause of vitamin instability. The type of trace mineral will influence its reactivity and more critically, the form that the trace mineral is presented in has an even more significant role to play in influencing vitamin stability. Well documented studies examining vitamin stability in mineral premixes containing inorganic sulphates demonstrate that inorganic minerals are detrimental to the stability of vitamins. Depending on the individual product however, the use of chelated minerals may not cause such a dramatic decrease. A recent study illustrates these effects nicely (Figure 3). The study, which examined Vitamin E function following short term inclusion in mineral premixes containing either inorganic or organic trace minerals demonstrates the importance of carefully choosing premix components. A clear difference can be noted in terms of the pro-oxidant potential of some of the mineral sources, further indicating the contrasting stabilities and thus reactivities of individual products.
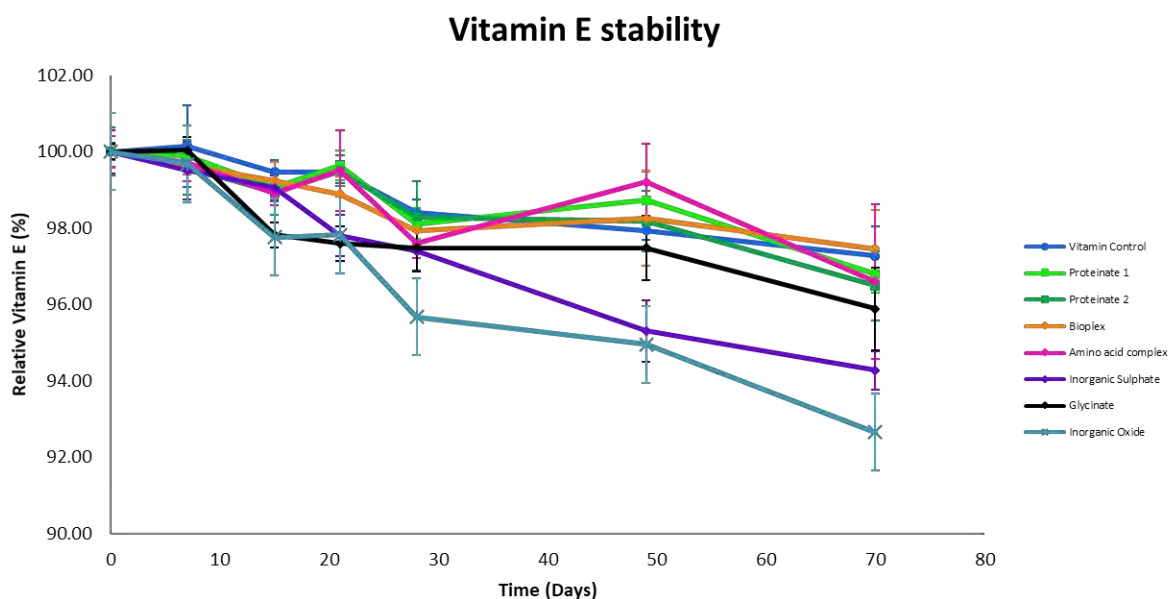
Figure 3. Relative activity of Vitamin E following exposure to organic (OTM) and Inorganic (ITM) sources of Fe^{2+}

Oxidation-reduction reactions have also been demonstrated to have negative impacts on labile amino acids such as methionine. In recent times, selenium derivatives of methionine (SeMet) have become commonplace in nutritional supplementation strategies as a vehicle to provide the essential trace element selenium. The distribution and accumulation of selenium in animal tissues depends greatly on the type of selenium supplement, with the way the element is presented playing a crucial role in its bioavailability and efficacy. The compounds available for use as Se supplements include the inorganic forms, sodium selenite and sodium selenate, along with organic sources such as selenium-enriched yeast (Se-Y). Additionally, newly developed chemically synthesised sources including L-SeMet and a newer hydroxy-analogue of selenomethionine (2-hydroxy-4-methylselenobutanoic acid, HMSeBA) have been approved for use as feed additives. Selenized yeast, in which selenium is taken up by the yeast during a controlled fermentation process, is the most researched source of selenium and widely regarded as being the most bioavailable organic selenium source for animal and human nutrition. One advantageous attribute of selenium yeast is the acknowledged stability of the selenoamino acids such as SeMet due to the inherent protection afforded them due to their incorporation into yeast protein. The use of chemically synthesised 'free' SeMet products such as L-SeMet as a dietary Se source is on the other hand potentially problematic. While methionine is readily oxidised, unprotected SeMet is even more susceptible to oxidation, thus representing an even more labile form of an already labile nutrient.

A recent VTM premix study (manuscript in preparation) comparing the stability of protein bound selenomethionine in selenium yeast with that of chemically synthesized free L-SeMet demonstrated clear differences in not only the stabilities of the selenomethionine sources but also demonstrated the differential impact of trace mineral form on SeMet stability (Figure 4.)
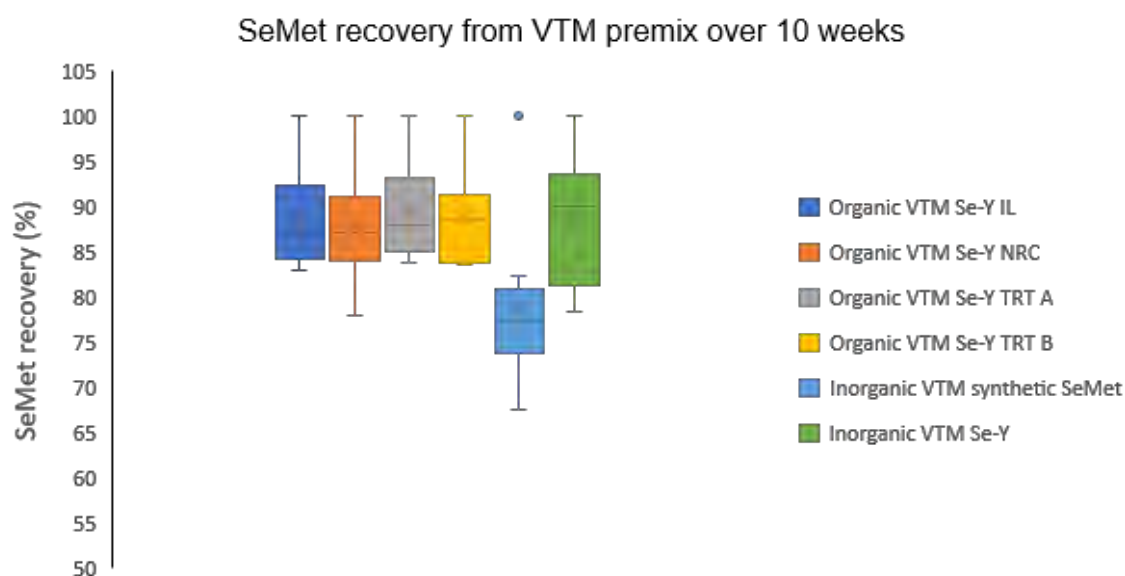
Figure 4. SeMet recovery from VTM premix

In this study the enhanced stability of the selenium yeast selenomethionine is apparent when compared to the extremely labile chemically synthesised selenomethionine source.

Overall it is clear that organic mineral chelates have far less potential for reactivity compared to inorganic mineral sources. However, organic trace mineral products with sub-optimal stabilities can result in inhibition of enzyme activity, induce vitamin instability and result in reduced antioxidant function. These effects can have negative consequences on not only feed quality but also on animal health and performance. Ultimately, diet formulators may well need to pay greater attention to their choice of mineral product to not only minimize the financial costs associated with any potential negative interactions but also to maximise animal wellbeing.

Conclusions

Despite the confusion and often contradictory information that exists, mineral chelation is a relatively straightforward process governed by some fundamental chemistry basics. By carefully considering factors important in mineral chelation, one can begin to distinguish between the products on the basis of their biological stabilities and thus biological bioavailability.

Ultimately, the stability of an OTM is of paramount importance to its bioavailability. During transit through the GI tract and as the pH decreases or acidifies, all OTMs are subjected to physiological forces which can result in the bound mineral complex dissociating and releasing free mineral ions. Organic trace minerals with optimized stability and bond strength will have far less potential for reactivity compared to inorganic sources. However, different forms of organic trace mineral will react differently and cause greater or less inhibition of enzyme activity, vitamin stability and antioxidant function depending on source.
Ultimately, diet formulators may well need to pay greater attention to their choice of individual component to minimize the financial costs associated with negative interactions which could be significant.

References

AAFCO. 1998. Official publication of the Association of American Feed Control Officials incorporated (Ed. Paul. M. Bachman). pp. 237-238.

Byrne, L.A. (2010) Analytical assessment of peptide-metal interactions and subsequent stability. Ph.D. Thesis. Dept. of Biology, National University of Ireland, Maynooth, Ireland.

Byrne, L.A., Hynes, M.J., Connolly, C.D. and Murphy, R.A. (2011) Analytical determination of apparent stability constants using a copper ion selective electrode. *Journal of Inorganic Biochemistry*, 105(12):1656-1661.

Byrne, L.A., Jacques, K.A. and Murphy, R.A. (2018)

Assessment of organic mineral chelate stability using computational and experimental approaches. Intl. Poult. Sci. Forum, Atlanta, GA, USA, 29-30 January, pg 90-91, 2018

Concarr, M.J., Lagoda, M., Byrne, L.A. and Murphy, R.A., (2016) The effect of mineral source and level on the activity of antioxidants. EAAP, 67th Annual Meeting, Belfast, UK 29 Aug - 2 Sep,

Martell, A. E. and Motekaitis, R. J., (1992) The Determination and Use of Stability Constants, 2nd Edition, VCH Publishers, Deerfield Beach, FL.

Murphy R.A. (2015) OTM bond strength, relative stability

Feedstuffs July

Murphy, R.A. (2016a) The influence of minerals on the stability of premix and feed components. Feed Navigator. http://www.feednavigator.com/Product-innovations/The-influence-of-minerals-on-the-stability-of-premix-and-feed-components

Murphy, R.A. (2016b) Mineral form's influence on enzyme supplementation response, http://www.wattagnet.com/articles/25983-mineral-forms-influence-on-enzyme-supplementation-response

Murphy, R.A. (2017) Organic trace minerals: enhanced stability ensures efficacy. *Pig International*, Sept., 14-18.

Murphy R.A. (2018a) Organic Trace Minerals: Optimised Stability Enhances Bioavailability International Animal Health Journal Vol 5 (2) 28-32

Murphy, R.A. (2018b) Minerals, Meals and Molecular Malnutrition: How Mineral Form can Impact Feed Quality and Cellular Health. International Animal Health Journal Vol 5 (1) 42-46

NIST Database 46 (2004) Critically selected stability constants of metal complexes. Texas A&M University College Station, TX

O'Rourke, R., Gaffney, M., Byrne, L. and Murphy, R. (2016) The effect of mineral source and level on the activity of exogenous phytase. 67th Annual Meeting of the European Federation of Animal Science (EAAP), Belfast, NI.

Santos, T., Connolly, C. and Murphy, R. (2015) Trace element inhibition of phytase activity. *Biological Trace Element Research*. 163(1-2): 255-65.

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The interaction of dietary protein on gut health, microbiota and animal performance

L'interaction des protéines alimentaires sur la santé intestinale, le microbiote et la performance animale

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Abstract

The dietary protein is fundamental for animal growth, health and performance. However, the digestibility of dietary protein could be affected by many factors such as animal enzymes production and activity dependent on age and disease. Also, anti-nutritional factors in feed ingredients like trypsin inhibitor and lectin in soybean and gossypol in cotton seeds can affect protein digestibility. The literature shows that 20% of crude protein in a corn/soybean diet is not digestible for monogastric animals. Recent studies have shown that undigested protein are fermented by microbiota to produce harmful metabolites that induces mucosa inflammation, poor digestion and performance. This issue seems to get more critical since the livestock producers are facing growing pressure of not using antibiotic growth promoters (AGPs) due to the concern that pathogenic bacteria are developing resistance to medicines applied in human health. The effect of dietary protein on microbiota and health and the manipulation of GIT microbiota by means of dietary protein nutrition will be discussed in this presentation. In addition, different methodologies will be addressed for evaluating solutions to gut health and performance.

Résumé

Les protéines alimentaires sont fondamentales pour la croissance, la santé et les performances des animaux. Cependant, leur digestibilité peut être influencée par de nombreux facteurs, tels que la production et l'activité enzymatiques en fonction de l'âge et de la maladie. De plus, des facteurs antinutritionnels dans les ingrédients alimentaires, comme l'inhibiteur de trypsine et la lectine dans le soya ainsi que le gossypol dans la graine de coton, peuvent nuire à la digestibilité des protéines. La littérature scientifique révèle que 20 % des protéines brutes d'un régime de maïs/soya ne sont pas digestibles pour les monogastriques. Des études récentes ont montré que les protéines non digérées sont fermentées par le microbiote pour produire des métabolites nocifs responsables d'une inflammation des muqueuses, de troubles digestifs et de mauvaises

performances. Cette question semble prendre de l'importance dans le contexte où la pression se fait de plus en plus forte pour que les éleveurs de bétail abandonnent les antibiotiques stimulateurs de croissance en raison de la crainte que des bactéries pathogènes acquièrent une résistance aux médicaments appliqués à la santé humaine. L'effet des protéines alimentaires sur le microbiote et la santé ainsi que la manipulation du microbiote du tractus gastrointestinal par les protéines alimentaires seront abordés dans cet exposé. En outre, il sera fait état de différentes méthodologies permettant d'évaluer les solutions en matière de santé intestinale et de performance animale.

Introduction

The protein is an essential nutrient for every animal diet. It is a source for growth, maintenance, immunity and production for all animals. Actually, every animal species have they own requirement for amino acids. The amino acids are classified as essential amino acids or non-essential amino acids. The essential amino acids are the one not synthetized *in vivo* and need to be supplier for the diet. There is another amino acids that is called non-essential, because they could be metabolize in some metabolic route, by in some situation of stress or disease, the increase in their demand could be optimized by diet. There is also the concept of "functional amino acids", for those that are fundamental key in specific metabolic route for cell surviving as arginine, cysteine, glutamine, glutamate, glycine, leucine, proline, and tryptophan which are known to improve the efficiency of utilization of dietary proteins in pigs (Kim and Wu, 2004; Li et al., 2007, Wang et al, 2008).

Normally in monogastric animals, the digestibility of proteins starts in the stomach and finish in the luminal enterocyte membrane. However, many factors can affect this digestibility of proteins. The amount of diet non-digested protein associated to endogenous proteins (from mucus and gut epithelial losses) could be fermented by the microbiota in large intestine and results in metabolites that could be an issue for the animal.

This presentation will review some important aspects of protein digestion, the factors that could affect it and the consequence of poor protein digestion for animal health and environmental.

Physiology of Protein Digestion

The digestion of proteins in monogastric animals starts in the stomach (acid phase), where the HCl and the pepsinogen are able to digest the big blocks of proteins in smaller particles. The second phase (neutral phase) is in small intestine where, at the basic pH, pancreas proteases (trypsin, chymotrypsin, elastases carboxypeptidases A and B) are able to reduce this big proteins parts in small peptides. However, the enterocytes, are just able to absorb di and tri-peptides and the last part of digestion of proteins is finalized by proteases present at mucosal brushed border membrane of enterocyte.

Most of the free amino acids, di and tri-peptides are absorbed in the small intestine via active transport, simple diffusion, and facilitated diffusion.

However, this process is very efficient, there is some factors that could affect the protein digestibility. For example, in young animals, like in piglets, the low capacity of gastric secretion

at birth may relate to immaturity of the parietal cells in piglets. The acidity of gastric contents in the post absorptive state is about pH 3 to 5 in milk-fed piglets during the early postnatal period due to low gastric secretory capacity and the high buffering capacity of sow's milk (Reza et al, 2013). As a result, in 14-day-old pigs reared by sows and in 30-day-old pigs weaned at 21 days of age, only 70% and 55% of dietary amino acids are deposited in tissue proteins, respectively (Wu et al., 2010).

In chickens and turkeys also the activities of pancreatic enzymes are limited in young animals and this will results in the non-digested protein that could be fermented in the large intestine.

The health status of animal will also contribute for more efficient or poor diet digestion in any animal, because of increase in the flow rate in the intestine, effect on pancreas and other gastrointestinal organ efficiency on digestion.

Another factor that could affect the digestibility of proteins are related to quality of cereals. Proteases inhibitor in raw materials as anti-trypsin factor or gossypol, mycotoxins and other anti-nutritional factors could also affect the efficiency of protein digestion.

The remaining protein from the digestion associated to the endogenous lost by the epithelial mucosa must be degraded to CO_2, NO, CO, H_2S, methane, H_2O, ammonia, urea, nitrate, and other nitrogenous metabolites (Wu et al., 2012, Li et al, 2017). Excretion of these products in urine and feces is a source of environmental pollution and can contribute to global climate changes.

Microbiota and Protein Fermentation in the intestine

However, the liver is the major site for metabolism of amino acids, the nutritional metabolism of amino acids in the intestine is important for normal gut function and intestinal mucosa growth;

Using genome it was possible identified 3499 metabolic reaction in the model of host/microbiota, these **1267 are unique to the microbiota** and **1142 are shared with the host** (Sridharan et al, 2014), showing the importante of microbiota in the metabolism of aminoacids.

Microbiota could use amino acids from feed to: 1- synthesis of protein; 2- conversion or fermentation for they metabolism; 3- De novo synthesis of other amino acids; 4- production of different metabolites. Most of these metabolites seems to be deleterious for the gut and induce inflammation.

During the fermentation process, similar to fiber fermentation, protein fermentation produces SCFA, but they are accompanied by branch-chained fatty acids (BCFA), derivate from branch-chained amino acids (Leucine, Isoleucine and Valine), ammonia, amines, hydrogen sulfide, phenol and indoles (Macfarlane et al, 1992);

Shift of microbiota have been observed in small intestine after diet rich in protein (Qiu et al, 2018). Moderate reduction on protein level at diet reduce the *Clostridiacea* and biogenic amine in the ileum of pigs with increase of occluding and claudin (Fan et al, 2017);

In health humans high-protein diet revealed no change in the proportions of *Clostridium* and *Bacteroides* genera, or sulfate-reducing bacterial species, although a reduction in the pool of *Bifidobacteria* genus and others was observed (Brink- worth et al. 2009; Duncan et al. 2007).

Milk-fed piglets fed with higher protein presented good health and performance but higher gastrointestinal pH and a lower lactobacillus-to-coliform ratio (Partanen and Mroz 1999).

These results showing a dysbiosis as a consequence of the protein fermentation in animal intestine and this will affect the gut health and animal performance.

Alternatives to control Inflammation and dysbiosis as consequence of protein fermentation on the gut

To control or avoid the fermentation of proteins in the gut is associated to use of good quality of ingredients, formulation based in digestible amino acids and not crude protein, use of enzymes associated to gut modifiers additives and control of animal health and well-being.

Final Remarks

The concept that the digestibility of proteins can affect animal performance and health is presented.

This is associated to physiological limitation on the animals, quality of raw materials or diet formulation.

The products of the protein fermentation cause inflammation on TGI and also impact the environmental.

The knowledge of this could help us to find solution for this issue and make the animal production more sustainable.

References

Li P, Yin YL, Li DF, Kim SW, Wu G. 2007. Amino acids and immune function. Br J Nutr 2007, 98:237–252.

Kim SW, Wu G. 2004. Dietary arginine supplementation enhances the growth of milk-fed young pigs. J Nutr, 134:625–630.

Wang JJ, Chen LX, Li P, Li XL, Zhou HJ, Wang FL, Li DF, Yin YL, Wu G: Gene expression is altered in piglet small intestine by weaning and dietary glutamine supplementation. J Nutr 2008, 138:1025–1032.

Wu G, Bazer FW, Burghardt RC, Johnson GA, Kim SW, Li XL, Satterfield MC, Spencer TE. 2010. Impacts of amino acid nutrition on pregnancy outcome in pigs: mechanisms and implications for swine production. J Anim Sci, 88:E195–E204.

Wu G, Meininger CJ. 2002. Regulation of nitric oxide synthesis by dietary factors. Annu Rev Nutr 2002, 22:61–86.

Li XL, Bazer FW, Gao HJ, Jobgen WJ, Johnson GA, Li P, McKnight JR, Satterfield MC, Spencer TE, Wu G. 2009. Amino acids and gaseous signaling. Amino Acids, 37:65–78.

Sridharan GV, Choi K, Klemashevich C, Wu C, Prabakaran D, Pan LB, Steinmeyer S, Mueller C, Yousofshahi M, Alaniz RC, Lee K, Jayaraman A. 2014. Prediction and quantification of bioactive microbiota metabolites in the mouse gut. Nat Commun 5:5492. doi:10.1038/ncomms6492

Macfarlane, G.T.; Allison, C.; Gibson, S.A.W.; Cummings, J.H. 1988. Contribution of the microflora to proteolysis in the human large intestine. *J. Appl. Bacteriol.*, *64*, 37–46.

Qiu, K.; Zhang, X.; Jiao, N.; Xu, D.; Huang, C.; Wang, Y.; Yin, J. 2018. Dietary protein level affects nutrient digestibility and ileal microbiota structure in growing pigs. *Anim. Sci. J.*, *89*, 537–546.

Fan, P.; Liu, P.; Song, P.; Chen, X.; Ma, X. 2017. Moderate dietary protein restriction alters the composition of gut microbiota and improves ileal barrier function in adult pig model. *Sci. Rep.*, *7*, 43412.

Partanen KH and Mroz Z 1999. Organic acids for performance enhancement in pig diets. Nutrition Research Reviews 12, 117–145.

Reza Rezaei, Weiwei Wang , Zhenlong Wu, Zhaolai Dai, Junjun Wang, Guoyao Wu 2013. Biochemical and physiological bases for utilization of dietary amino acids by young Pigs. Journal of Animal Science and Biotechnology, 4:7.

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From human research to animal research and application: How do we make the leap?

De la recherche humaine à la recherche animale et son application : comment faire le saut?

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Abstract

Advances in animal and human research are leading to marked improvements in health, performance, efficiency, and wellbeing. Interestingly, human research is at the forefront of many nutritional breakthroughs. Inter-relationships between dietary nutrients, the gut microbiome, immunity, psychological response, and health are being investigated. Recent discoveries have identified associations between sources of dietary nutrients, that alter nutrient absorption and status, with subsequent effects on gut microbiome and integrity which further affect immunity, inflammation, and possible susceptibility to neurological disorders, such as Autism Spectrum Disorder (ASD).

Human scientists are working to determine the cause of increased developmental disabilities and more specifically ASD in children (Boyle et al., 2011). Interestingly, the Center for Disease Control and Prevention estimates that about 1 in 59 children will be identified with some level of ASD (CDC, 2020). Globally, this is a growing problem and relatively recent human and animal research may have identified key factors influencing its establishment. A negative relationship between folic acid (a zinc uptake antagonist) and zinc supplementation may be at the root of altered composition of gastro-intestinal (GI) microbiota, leading to increased GI permeability and brain neuroinflammation.

Remarkably, microbiota composition, gut pathology, and inflammatory cytokine levels were partially rescued upon supplementation with zinc amino-acid complexes (ZnAA).

This presentation will tie together findings in human and animal research to establish a clearer understanding of the role nutrition plays in physical and psychological wellbeing of man and animal.

Résumé

Les progrès de la recherche sur les animaux et les humains entraînent des améliorations marquées en matière de santé, de performance, d'efficacité et de bien-être. Les travaux portant sur l'humain sont au centre de nombreuses percées dans le domaine de la nutrition. Les relations entre les éléments nutritifs, le microbiome intestinal, l'immunité, les réactions psychologiques et la santé font l'objet d'études. Des observations récentes ont permis d'identifier des liens entre les sources d'éléments nutritifs qui modifient l'absorption des nutriments et l'équilibre nutritif, entraînant des effets sur le microbiome et l'intégrité de l'intestin qui interviennent ultérieurement sur l'immunité, l'inflammation et une éventuelle susceptibilité aux troubles neurologiques, tels que les troubles du spectre de l'autisme (TSA).

Les scientifiques cherchent à déterminer la cause de l'augmentation des troubles du développement et, plus particulièrement, des TSA chez les enfants (Boyle *et al*., 2011). Le Center for Disease Control and Prevention estime qu'environ 1 enfant sur 59 sera atteint d'un TSA, à un niveau ou un autre (CDC, 2020). À l'échelle mondiale, il s'agit d'un problème croissant, et des travaux relativement récents sur l'humain et l'animal pourraient avoir permis d'identifier les facteurs déterminants pour sa progression. Une relation négative entre l'acide folique (un antagoniste de l'absorption du zinc) et la supplémentation en zinc pourrait être à l'origine d'une modification de la composition du microbiote gastro-intestinal (GI), entraînant une augmentation de la perméabilité GI et une neuroinflammation cérébrale.

Fait à noter, la composition du microbiote, la pathologie intestinale et les niveaux de cytokines inflammatoires ont été partiellement normalisés après la supplémentation en complexes zinc-acides aminés (ZnAA).

Cet exposé fera le lien entre les résultats de la recherche sur l'humain et l'animal pour mieux expliquer le rôle que joue la nutrition dans le bien-être physique et psychologique de l'homme et de l'animal.

Introduction

Trace minerals are essential elements in the maintenance of life for man and animal. However, what is not well understood or appreciated is how absorption and retention of these minerals can affect animal and human wellbeing, psychological status, health, and performance. While required in small amounts, just milligrams per day, trace minerals play critical roles as catalytic, regulatory, and structural components in multiple enzyme and protein systems. Zinc, for example, is a key component in over 300 enzyme systems and a structural part of over 3000 proteins. Yet as a "type-II nutrient", zinc must be consumed on a daily basis to maintain normal bodily function. While

trace minerals are key to cellular functions, their role prior to absorption, in maintenance and growth of the gut microbiome, is also important. Mineral source, amount and presence of dietary components, such as fiber, phytase, or even folic acid may lead to imbalances and/or insufficiencies, resulting in microbiome alteration with concomitant effects on health and neural function (Sauer and Grabrucker, 2019). Interestingly, as much as 20% of ingested zinc may be required by intestinal bacteria for growth and function (Smith et al., 1972). An insufficient intake of zinc has been linked to depression, mental lethargy, and cognitive impairment in human and animal models (Figure 1).

Mineral Absorption

It is well established that both macro and trace minerals are capable of antagonizing absorption and retention of other minerals (Figure 2; NRC, 2001). The interrelationship between minerals and their requirements emphasize the importance of achieving a proper balance between amount and bio-efficacy. For example, when man or animal with normal zinc status consume excess zinc, the storage protein metallothionine is upregulated in intestinal enterocytes. This is a normal response, as metallothionine will hold absorbed zinc until it is either further moved into circulation or lost back into the gut for excretion via cellular turnover. The challenge is that metallothionine has a greater affinity for copper than zinc, thus copper movement into circulation may be antagonized by high zinc intake. The balance between zinc and copper is important in establishment and maintenance of gastro-intestinal (GI) microbiota and gut epithelial integrity, along with the potential for production of inflammatory cytokines (Sauer and Grabrucker, 2019). In a review, Vela et al. (2015) reported low levels of zinc and elevated copper have been linked to alterations in GI microbiota, increased intestinal epithelium permeability, and immune system abnormalities- all increasing the severity of symptoms associated with autism spectrum disorder (ASD). It is well established that zinc plays a key role in these systems and mothers consuming an insufficient supply of zinc are at greater risk for GI tract dysbiosis, with an increased risk of skin challenges, immune depression, psychological depression, and higher risk of birth defects.

Fiber, phytase (Figure 3), and the presence of divalent cations such as calcium, iron, and copper are well established as potential antagonists to mineral absorption from the GI tract, specifically zinc, having been shown to reduce mineral availability in many species of animals and man (NRC, 2005). In humans, it is common for women to consume calcium supplements to mitigate the effects of osteoporosis or drink water naturally high in calcium. Similarly, women often take iron supplements to prevent anemia and to maintain sufficient red blood cell numbers during pregnancy to ensure proper fetal development. However, supplements meant to aid in offspring health and wellbeing may not always be as they seem. Supplementation of pregnant women with iron and folate is a common practice for prevention of gestational anemia and fetal neural tube defects. Work by Ghishan et al. (1986) demonstrated that zinc and folic acid have a mutually inhibitory effect at the intestinal level. This means that women and/or animals consuming folic acid may be at greater risk for zinc insufficiency, thus suffering subsequent effects of altered mineral status, with potential unfortunate effects on their offspring.

Early works at Zinpro Corporation found that sheep consuming a daily allocation of folic acid and zinc demonstrated a marked decline in circulating zinc status. These findings led us to further

investigate the effects of folate supplementation on zinc retention. Our work, and that of Milne et al. (1984) demonstrated that folic acid supplementation resulted in increased fecal zinc excretion and a significant decline in urinary zinc, indicating a reduction in zinc retention and utilization. These findings are problematic, as Hagmeyer et al. (2015) reported that insufficient zinc intake/status is not only associated with skin lesions, growth retardation, poor appetite, delayed wound healing, cell-mediated immune dysfunction, and abnormal neurosensory changes, additionally, behavioral alterations have been consistently reported. Interestingly, recent findings in humans have documented an association between folate consumption, zinc insufficiency and depression, emotional instability, increased anxiety and aggression, irritability, and defects in social behavior. It is important to note that low serum zinc levels have been reported in Autism Spectrum Disorder (ASD), Attention Deficit Hyperactivity Disorder (ADHD), and mood disorders such as depression and schizophrenia (Hagmeyer et al., 2015).

Epigenetic Effects

Epigenetic effects are alterations in heritable phenotype without changes to the DNA sequence. For example, subclinical zinc deficiency in pregnant and lactating mothers is a possible cause for an immature and dysfunctional intestine in newborns (Vela et al., 2015). In a review, Vela et al. (2015) reported that maternal zinc status had teratogenic and pathological effects leading to developmental and functional effects in man and animal. Research indicated that low maternal zinc can result in impaired zinc absorption, leading to imbalance in zinc and copper, and behavior alterations in brain development in offspring.

Unfortunately, mineral insufficiency creates a cascade of effects that are not easily delineated. For example, insufficient zinc often leads to an alteration in GI tract permeability, i.e. gut leakage, which results in inflammation and localization of inflammatory cytokines with immune cell migration. At the same time, GI microbiota may begin to shift at the phylum and genus level, altering digestive functionality, yield of end products, and disruption of communication between the gut and central nervous system (Strati et al., 2017). It is well established that individuals with ASD often suffer with GI co-morbidities, as a result of altered gut microbiota. Thus, maternal nutrition has direct effects on GI development, establishment of a normal microbiota, and GI communication with the brain in offspring. Notably, early works at Zinpro Corporation showed that chicken eggs injected with zinc methionine complex not only survived to hatch, but also showed marked improvements in GI tract development and function (Figures 4 & 5; Ferket, NCSU). While the chicks had morphological improvements in GI epithelial integrity and function, these changes resulted in > 7% enhancement in body weight growth through 14 days-of-age.

Similarly, researchers found that feeding sows zinc, as zinc amino acid complex (ZnAA), during the last trimester of pregnancy resulted in improved intestinal development of baby pigs (Figure 6; Caine et al., 2009). Offspring of sows fed ZnAA had increased villous height and villus height:crypt depth ratio in the jejunum, and greater goblet cell numbers in the ileum. These works demonstrate that maternal nutrition plays key roles in offspring morphological development and may also alter neural development and function.

Mineral Form and Bioavailability

Maternal nutrition is only part of the story, as mineral source is also a key component in meeting the nutritional requirements of man and animals. In general, inorganic minerals are less bioavailable and metabolically active than "organic" forms of trace minerals. A mineral is deemed organic when it is complexed or chelated to an amino acid, protein fragment, or other carbon-containing material. Unfortunately, much confusion and misinformation exists in regard to the true chemical structure and bio-efficacy of various organic mineral sources.

With regard to this particular discussion on mineral supplementation and its impact on GI tract integrity and prevention of GI microbiota dybiosis, Sauer and Grabrucker (2019) reported ZnAA was taken up by cells, not through classical zinc importer proteins, but rather by amino acid transporters. They noted ZnAA showed significant advantages over inorganic mineral salt supplements, as they did not compete with other metals for GI epithelium transporters and were less accessible for folic acid or phytic acid antagonism. This work is supported by earlier work demonstrating ZnAA was markedly less influenced by dietary antagonists, and therefore more bioavailable for absorption (Wedekind et al., 1994).

In unpublished work utilizing zinc knockout mice, workers at Vanderbilt University were able to create an ASD-type disorder in mouse pups by controlling maternal zinc supplementation. The pups were identified to have alterations in GI zinc and copper balance, impaired zinc uptake, and microbiome dysbiosis. Interestingly, expression of ASD in the pups was then reversed through supplementation with ZnAA. These findings were not totally unexpected, as work by Sauer and Grabrucker (2019) demonstrated that ZnAA was absorbed differently than inorganic zinc sources, and positively impacted GI permeability, GI microbiota, and reduced neuroinflammatory markers.

Conclusion

Human and animal research demonstrates that maternal mineral nutrition affects not only maternal health and wellbeing, but also that of her offspring. Maternal mineral insufficiencies have been shown to elicit epigenetic effects on offspring, impacting GI tract development and integrity, microbiota diversity, behavior impairment, and susceptibility to ASD, ADHD, and depression. Selection of bioavailable mineral sources, such as metal amino acid complexes, has shown to improve GI tract integrity, re-establish normal gut microbiota, and by enhancing mineral status improve neural function, behavior, and sense of wellbeing.
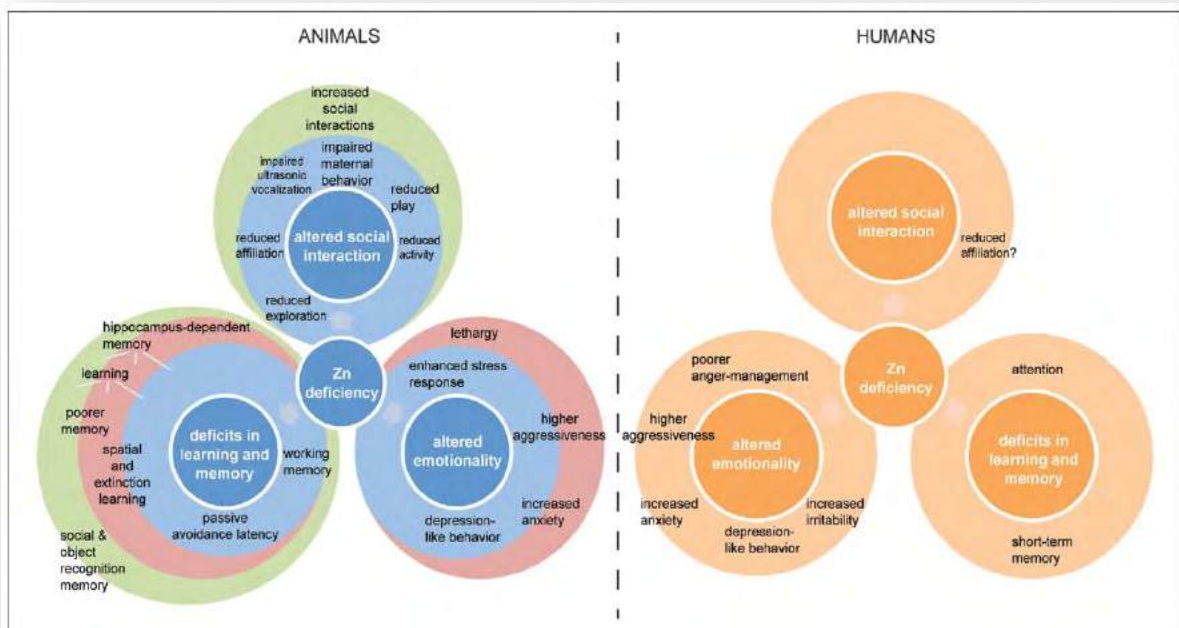
Figure 1. Comparative overview of behavioral alterations associated with zinc deficiency in animals and humans.

Hagmeyer et al. 2015. Front. In Behav. Neurosci. 8:443
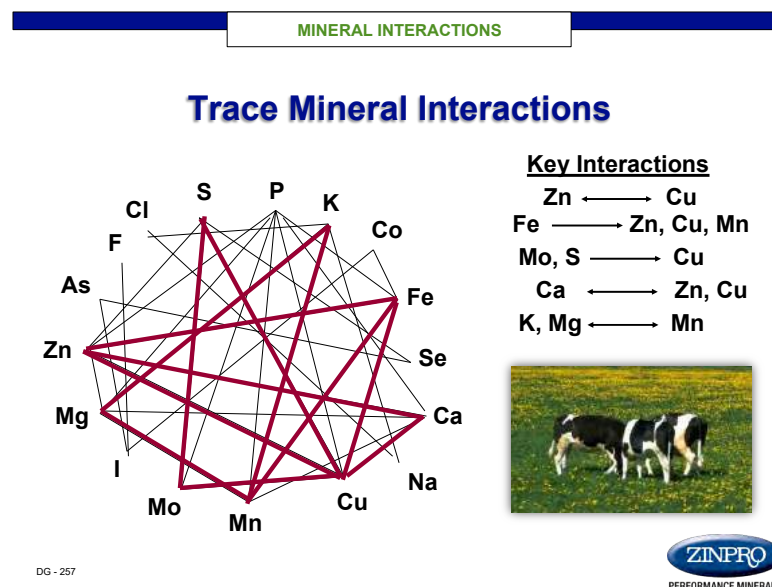
Figure 2. Macro and trace mineral interactions in man and animals.
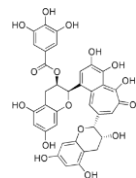
Figure 3. Structure of phytic acid and its influence on mineral complexation and bioavailability in diets.
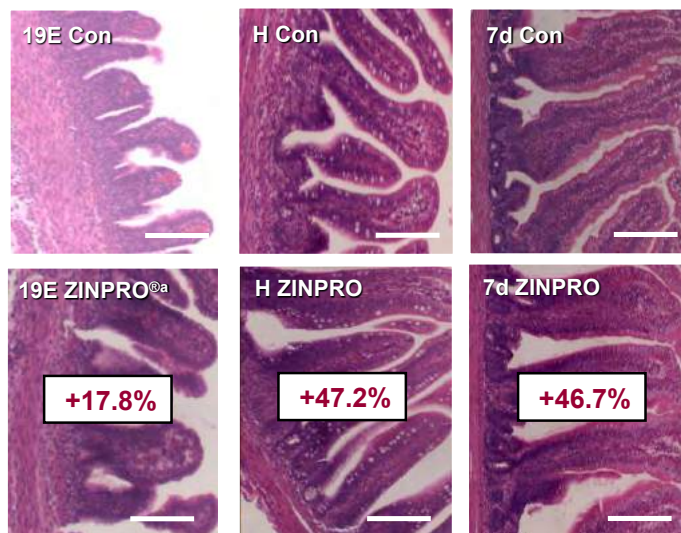
Figure 4. Impact of *in ovo* feeding with ZINPRO® zinc methionine on intestinal integrity of chicks.
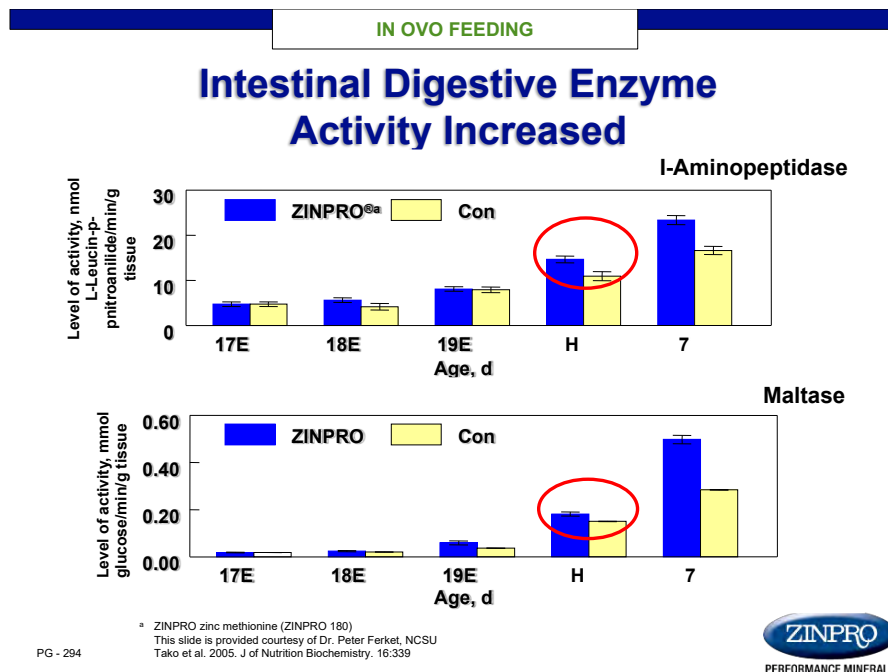
Figure 5. Impact of *in ovo* feeding with ZINPRO® zinc methionine on intestinal digestive integrity of chicks.
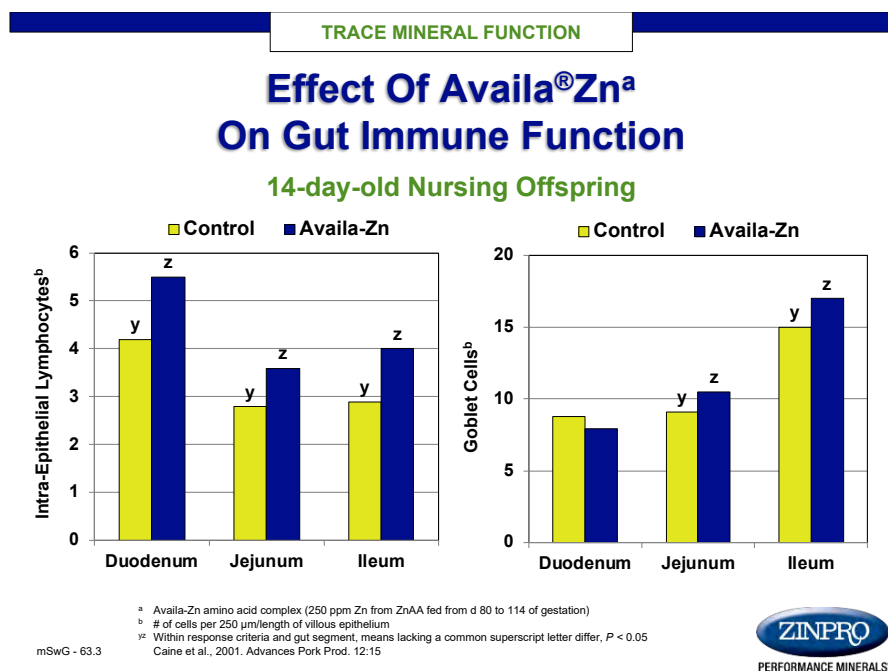
Figure 6. GI tract integrity and functional effects in newborn baby pigs after late gestation maternal supplementation with zinc amino acid complex.

Bioavailability[a] Of Zinc Amino Acid Complex[b]

RELATIVE TO ZINC FROM $ZnSO_4$
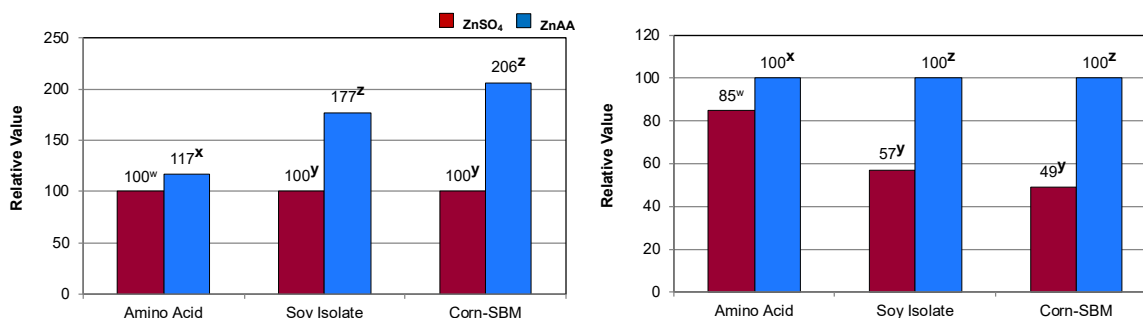
a Bioavailability estimated using multiple regression analyses of total tibia Zn in chicks; $ZnSO_4$ set to 100

b ZINPRO zinc methionine

wx Means lacking a common superscript letter differ, $P < 0.05$

yz Within diet type, means lacking a common superscript letter differ, $P < 0.01$

Wedekind et al., 1992. J. Anim. Sci. 70:178-187

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Figure 7. Impact of zinc source and antagonists on relative bioavailability.

References

Boyle, C. A., S. Boulet, L. Schieve, R. A. Cohen, S. J. Blumberg, M. Yeargin-Allsopp, S. Visser, and M. D. Kogan. 2011. Trends in the Prevalence of Developmental Disabilities in US Children, 1997–2008. Pediatrics. 127(6):1034-1042.

Caine, W. R., B. U. Metzler-Zebeli, M. McFall. 2009. Supplementation of diets for gestating sows with zinc amino acid complex and gastric intubation of suckling pigs with zinc methione on mineral status, intestinal morphology and bacgerial translocation in lipopolysaccharide-challenged early weaned pigs. Res. Vet. Sci. 86(3):289-296.

CDC, 2020. Data and Statistics on Autism Spectrum Disorder. https://www.cdc.gov/ncbddd/autism/data.html

Ghishan, F. K., H. M. Said, P. C. Wilson, J. E. Murrell and H. L. Greene. 1986. Intestinal transport of zinc and folic acid: a mutual inhibitory effect. Am. J. Of Clin. Nutr. 43:258-262.

Hagmeyer, S., J. C. Haderspeck, and A. Martin. 2015. Behavioral impairments in animal models for zinc deficiency. Front. Behav. Neurosci. 8:443. http://www.frontiersin.org/Behavioral_Neuroscience

Milne, D. B., W. K. Canfield, J. R. Mahalko, and H. H. Sandstead. 1984. Effect of oral folic acid supplements on zinc, copper, and iron absorption and excretion. Am. J. Clin. Nutr. 39:535-539.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle. 7th ed. Natl. Acad. Press, Washington, DC.

National Research Council. 2005. Mineral Tolerances of Domestic Animals. 2nd rev. ed. Nat. Acad. Press, Washington DC.

Sauer, A. K., and A. M. Grabrucker. 2019. Zinc deficiency during pregnancy leads to altered microbiome and elevated inflammatory markers in mice. Front. Neurosci. 13:1295.

Smith, J. C. Jr., E. G. Daniel, L. D. McBean, F. S. Doft, and J. A. Halsted. 1972. Effect of microorganisms on zinc metabolism using germfree and conventional rats. J. Nutr. 102:711–719.

Vela, G., P. Stark, M. Socha, A. K. Sauer, S. Hagmeyer, and A. M. Grabrucker. 2015. Zinc in gut-brain interaction in autism and neurological disorders. Neural Plast. 2015:972791

Wedekind, K. A, A. J. Lewis, M. A. Giesemann, and P. S. Miller. 1994. Bioavailability of zinc from inorganic and organic sources for pigs fed corn-soybean meal diets. J. Anim. Sci. 72:2681-2689.

Conference

Colloque

Immunometabolism: The Potential Cause of and Solution to Our Most Pressing Poultry Problems in Health and Infectious Disease

Immunométabolisme : la cause potentielle et la solution de nos problèmes avicoles les plus urgents en matière de santé et de maladies infectieuses

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Abstract

Poultry, and chicken specifically, have been a research model organism for decades. Much of our most fundamental and consequential discoveries in immunology and disease have come from the study of poultry. These discoveries cross the research spectrum from oncogenes to B-cells. At the same time chickens have been aggressively, and successfully, bred for greater growth and feed efficiency. While this breeding has been an indisputable success in terms of food animal production, it has had consequences in terms of emerging and re-emerging disease challenges in poultry. These challenges have a common theme in the form of the recent research perspective called immunometabolism. Immunometabolism refers to the interconnectedness of immune responses and metabolic processes. We can use an immunometabolic perspective to better understand poultry disease, both infectious and not. Immunometabolism has pointed to potential mechanisms of woody breast, the tolerance of chicken toward *Salmonella*, and the devastating effects of coccidiosis and necrotic enteritis. The challenges that we face in growing poultry ever more efficiently is coupled with greater restrictions on how poultry production is managed. These challenges of immunometabolism are also an opportunity. An integrated metabolic and immunologic perspective allows us to more fully understand disease pathogenesis and the mechanism of action of effective disease interventions. Importantly, it also opens up an entirely new world of possible solutions, nutritional and metabolic solutions, to the pressing health and disease problems in poultry.

Résumé

La volaille, et plus particulièrement le poulet, est un organisme modèle pour la recherche depuis des décennies. La plupart de nos découvertes les plus fondamentales et les plus importantes en matière d'immunologie et de maladies proviennent de l'étude de la volaille. Ces découvertes couvrent tout le spectre de la recherche, des oncogènes aux cellules B. En même temps, les poulets ont été vigoureusement sélectionnés, avec succès d'ailleurs, pour améliorer la croissance et l'efficacité alimentaire. Si ce travail de sélection génétique est une réussite incontestable au plan de la production animale pour l'alimentation humaine, il a eu des conséquences sur les problèmes de maladies émergentes et réémergentes chez les volailles. Ces défis ont un thème commun sous la forme de la récente perspective de recherche appelée immunométabolisme. L'immunométabolisme fait référence à l'interconnexion des réponses immunitaires et des processus métaboliques. Nous pouvons utiliser une perspective immunométabolique pour mieux comprendre les maladies des volailles, qu'elles soient infectieuses ou non. L'immunométabolisme a mis en évidence les mécanismes potentiels qui interviennent dans le « woody breast », la tolérance du poulet à *Salmonella* et les effets dévastateurs de la coccidiose et de l'entérite nécrotique. Les défis à relever pour produire la volaille de manière toujours plus efficace se doublent de restrictions plus importantes quant à la gestion de la production avicole. Ces défis de l'immunométabolisme sont aussi des possibilités. Une perspective métabolique et immunologique intégrée nous permet de mieux comprendre la pathogenèse et le mode d'action des interventions efficaces de lutte contre la maladie. Fait important, elle ouvre également un monde entièrement nouveau de solutions possibles, nutritionnelles et métaboliques, aux problèmes urgents de santé et de maladie chez les volailles.

Poultry Immunity

The poultry immune system is in many ways the "standard" vertebrate immune system, which is what makes it such a good model for immune research, though there are several key differences when compared to other agricultural species. Indeed, much of our fundamental understanding of immunology has come from the study of poultry, specifically chickens. Discoveries including retroviruses, oncogenes, interferon, vaccine development and B-cells have come from chicken studies (Stern, 2005; Taylor Jr and McCorkle Jr, 2009; Weiss, 1998). The vertebrate immune system is comprised of two branches, the evolutionarily more ancient innate immune system and the vertebrate-evolved adaptive immune system (Chaplin, 2010). The innate immune system involves non-specific barriers and responses, barriers such as skin and mucus, and responses such as inflammation and temperature increase (fever). The innate response is very rapid to immediate, and generally non-specific, recognizing patterns associated with pathogens rather than individual microbes. The adaptive immune system is a more recent evolutionary development, is cell-mediated or humoral, and extremely specific, though slower to respond to antigen insult. Receptors, initially low affinity, found on T- and B-cells result in stimulation and cellular expansion leading to an antigen-specific respond that can distinguish between close serovars. The adaptive immune system can generate an active response or tolerance to commensals and maintains an immune memory for subsequent antigen exposure.

Immunometabolism

Historically, nutrition and/or metabolism and immunology were treated as distinct research disciplines, and the significant cross-talk between the two was underestimated. With the emerging field of immunometabolism, where immunity and metabolism are considered part of an integrated whole, the connections are beginning to be unraveled (Mathis and Shoelson, 2011). There has been a veritable explosion in research related to immunometabolism in recent years. A Google Scholar search for "immunometabolism" conducted in February 2020 for papers published between 1900-2010 generated 361 citations, while that same search for papers published between 2010-2020 generated 5,850 citations.

The impact of nutrition on immune potential has been known for decades, and nutritional studies in poultry that determined the limiting nutrients in effective immune responses allowed us to understand the nutritional building blocks of a proper immune system (Klasing, 2007). One aspect of the nutrition-immune dynamic that was clearly shown was that *engaging* the innate immune system is very energetically costly, though it is not costly to establish the system in the first place, while the adaptive immune system *response* is energetically efficient, though costly to establish the system beforehand (Klasing, 2007). The innate response causes an increase in thermogenesis, an invasion of effector cells, increase in blood supply, generation of expanded innate immune cells, production of cytokines and chemokines, among other changes, all placing significant energy and resource demands on the organism requiring enhanced metabolism and nutrient consumption. The adaptive immune system is already, in the main, established before an immune insult, a repertoire of antigen presenting cells, T-, and B-cells are present and ready to respond, for example. When the antigen receptor system is engaged a specific clonal expansion of B-cells and the engagement of a controlled T-cell response places less of a metabolic burden on the host. Following an initial adaptive response and the establishment of immune memory toward the antigen, there is almost no nutritional consequences to a secondary response to that same antigen (McDade et al., 2016). The activation of an immune cell, such as a macrophage or T-cell, has metabolic consequences for that cell. The reverse is also true; the alteration of metabolism in an immune cell can alter that cells immune response or potential. Shifting a macrophage from a predominantly glycolytic metabolic state to a predominantly oxidative phosphorylation-based metabolism shifts the cell from a pro-inflammatory M1 phenotype to an anti-inflammatory M2 phenotype (He and Carter, 2015). This phenomenon is also true of T-cells, shifting metabolism can result in either an effector T-cell (glycolytic) or T-regulatory (T-reg) cell (oxidative phosphorylation) (Mockler et al., 2014).

The Modern Broiler and Disease

Breeding and Immunometabolism
The modern broiler (meat production) chicken has been aggressively, selectively bred for feed efficiency for many decades. When Cecile Steele serendipitously started the United States broiler industry on her farm in Sussex County, Delaware (Williams, 1998), a chicken grew to 1.13 kg in 112 days, now a modern commercial broiler grows to 2.84 kg in 47 days (National Chicken Council). The modern broilers feed to meat gain ratio is now 1.8 kg-to-kg, the most efficient terrestrial agricultural animal. This fantastic increase in productivity and efficiency, 80% of which

is due to genetic selection (Havenstein et al., 2003), has had an impact on the immune system of these birds, in addition to their metabolism.

Coccidiosis

The chicken disease coccidiosis, caused by the *Eimeria* parasite, is one of the major production loss diseases in the poultry industry. The total cost to the industry is estimated at $2.4 billion U.S.D (Quiroz-Castañeda and Dantán-González, 2015). *Eimeria* enters orally and invades and destroys the epithelium of the chicken gut, this leads to reduced feed intake, bloody diarrhea, reduced weight gain and can proceed to death. Different species of *Eimeria* preferentially invade different segments of the gut, *E. acervulina* develops in the duodenum, *E. maxima* and *E. mitis* develop in the middle part of the small intestine, *E. tenella* develops in the caeca, *E. brunetti* develops in the caeca and lower intestine, and *E. necatrix* develops in the small intestine (Quiroz-Castañeda and Dantán-González, 2015). A vaccine for coccidiosis is available; it incorporates various species of *Eimeria* at a low dose to expose the immune system to the parasite, resulting in subsequent protection. Groups, including Wang, et al., have shown that even the *Eimeria* containing vaccine has an impact on bird weight gain (Wang et al., 2019). Our group compared the heritage breed Athens-Canadian Random Bred (ACRB) broiler chicken that represents 1957 genetics with a modern broiler chicken following coccidia vaccination and found that that in the ACRB there was an induction of apoptosis / inhibition of cell growth, and in the modern broiler, there was an induction of cell growth related signaling. The apoptosis response may represent an attempt to restrict pathogen growth and spread, as *Eimeria* is an intracellular pathogen. Following a pathogenic dose of *Eimeria* the ACRB again induced apoptosis and initiated a glycolytic response while the modern broilers did not show these responses. These results highlight the immunometabolic differences following pathogen challenge due to the differing genetics of these two lines of chickens.

Salmonella

In general, chickens are refractory in their immune response to the *Salmonella* species of greatest health concern to humans (Kogut and Arsenault, 2017). This results in a foodborne illness problem as *Salmonella* harboured in the chicken gut or organs can contaminate poultry products and result in human illness. Our group and others have determined that if *Salmonella* is given early in a chick's life they will generate an inflammatory response to the bacteria. This response manifests in several ways: an interleukin (IL)-6 to TGF-B transition around day 4, induction of Wnt signaling, and the activation of NFκB (Kogut and Arsenault, 2015). After 4 to 7 days post-hatch, a transition occurs in chickens where anti-inflammatory IL-10 is expressed and there is an increase in T-reg cells, both resulting in reduced immune response (Shanmugasundaram et al., 2015). We determined that one mechanism of this transition is the inhibition of glycogen synthase kinase 3β (GSK3β) and thus glycolysis by *Salmonella* infection (Kogut et al., 2014). With GSK3β inhibited, there is a decrease in expression of pro-inflammatory cytokines and an increase in anti-inflammatory cytokine IL-10. The result being a tolerance of *Salmonella* in the gut of infected chickens.

Table 1. Summary of the immunometabolic changes occurring in the chick following exposure to *Salmonella*. Adapted from (Kogut and Arsenault, 2017)

Characteristic	Early (4-48 hours)	Late (4-14 days)
Immune cells	Heterophils, macrophages	Tregs
Transcription factor activation	NF-κB	NFAT, Smad1-3
Cytokine mRNA expression	IL-1β, IL-6 IFN-γ	IL-10, TGF-β4
Metabolism	Anabolic	Catabolic
Characteristic metabolic processes	Glycolysis, protein synthesis	Oxidative phosphorylation
Energy balance	AMP:**ATP**	**AMP**:ATP
Regulatory enzyme activity	mTORC1 phosphorylation	AMPKα phosphorylation
Tissue phenotype	Pro-inflammatory	Anti-inflammatory (Tolerance)

Wooden Breast

Wooden breast is a myopathy that involves the pectoral muscle of commercial broiler chickens (Papah et al., 2018). The disorder is characterized by abnormally firm muscle, resulting in an undesirable consistency of the meat, which affects meat quality traits. Expression profiling of the muscle tissue comparing wooden breast to normal breast muscle tissue has shown a variety of immune and metabolic changes occurring in the abnormal ("wooden") muscle cells (Papah et al., 2018). These changes include an increase in inflammatory response, metabolic dysregulation of lipids and fatty acids and connective tissue remodeling abnormalities. In addition to breeding for rapid growth and feed efficiency, breeders in the United States have selected broiler chickens for the development of large breast muscle, as these cuts of meat are the most desirable in the U.S. market. This puts the pectoral muscle under significant metabolic and physical stress as it rapidly grows, growing out of proportion to the rest of the chicken body. As a result, there are alterations in immune responses to this metabolic program in some chicken strains resulting in abnormal muscle deposition.

The Challenge and the Opportunity

In both animal production and human health, the interactions between immunity and metabolism can appear to be a significant challenge. In humans, issues with obesity can lead to chronic immunological diseases, including autoimmune diseases. The demands of animal agriculture

production, greater yield with fewer inputs, places significant metabolic strain on animals, thus impacting their immune potential and susceptibility to disease. However, this immune-metabolism interplay can also be viewed as an opportunity. The dichotomy that once stated that the solutions to growth and to disease are distinct no longer apply. Using the knowledge we are gaining in immunometabolism we can expand our intervention repertoire to include solutions across the spectrum from nutrition changes to drugs. To date, the greatest advances using this perspective have come in cancer research. Tumors switch their metabolic program as they grow and divide uncontrollably, targeting this metabolic program can aid in the treatment of several types of cancer. Effector and helper T-cells can be inhibited by 2-Deoxy-D-glucose [2DG] or rapamycin by inhibiting glycolysis or mTOR, respectively (Mockler et al., 2014). These approaches may be applicable to infectious or metabolic disease such as activating an immune response with etomoxir, which inhibits the development of Tregs or metformin, which activates lipid oxidation. In animal production, with nearly total control of the diet formulations, we may be able to feed-in proper immune potential or feed-out excess inflammation in certain circumstances. Our research on postbiotics have shown that feeding the beneficial metabolites of a probiotic fermentate can modulate the gut immune response, not diminish or stimulate, to improve growth and resistance to pathogen challenge (Johnson et al., 2019). Thus, rather than the extreme solution advocated by some to return to slow growth, inefficient animal production systems, a nutritional or feed additive corrective to the current challenges may allow us to keep the dramatic gains we have attained in animal production while further improving the health, wellbeing and disease resistance of our domestic animals.

References

Chaplin, D.D. (2010). Overview of the immune response. J. Allergy Clin. Immunol. *125*, S3–S23.

Havenstein, G., Ferket, P., and Qureshi, M. (2003). Growth, livability, and feed conversion of 1957 versus 2001 broilers when fed representative 1957 and 2001 broiler diets. Poult. Sci. *82*, 1500–1508.

He, C., and Carter, A.B. (2015). The Metabolic Prospective and Redox Regulation of Macrophage Polarization. J. Clin. Cell. Immunol. *6*, 371.

Johnson, C.N., Kogut, M.H., Genovese, K., He, H., Kazemi, S., and Arsenault, R.J. (2019). Administration of a Postbiotic Causes Immunomodulatory Responses in Broiler Gut and Reduces Disease Pathogenesis Following Challenge. Microorganisms *7*, 268.

Klasing, K.C. (2007). Nutrition and the immune system. Br. Poult. Sci. *48*, 525–537.

Kogut, M.H., and Arsenault, R.J. (2015). A Role for the Non-Canonical Wnt-β-Catenin and TGF-β Signaling Pathways in the Induction of Tolerance during the Establishment of a Salmonella enterica Serovar Enteritidis Persistent Cecal Infection in Chickens. Front. Vet. Sci. *2*.

Kogut, M.H., and Arsenault, R.J. (2017). Immunometabolic Phenotype Alterations Associated with the Induction of Disease Tolerance and Persistent Asymptomatic Infection of Salmonella in the Chicken Intestine. Front. Immunol. *8*.

Kogut, M.H., Swaggerty, C.L., Chiang, H.-I., Genovese, K.J., He, H., Zhou, H., and Arsenault, R.J. (2014). Critical role of glycogen synthase kinase-3β in regulating the avian heterophil response to Salmonella enterica serovar Enteritidis. Vet. Infect. Dis. *1*, 10.

Mathis, D., and Shoelson, S.E. (2011). Immunometabolism: an emerging frontier. Nat. Rev. Immunol. *11*, 81–83.

McDade, T.W., Georgiev, A.V., and Kuzawa, C.W. (2016). Trade-offs between acquired and innate immune defenses in humans. Evol. Med. Public Health *2016*, 1–16.

Mockler, M.B., Conroy, M.J., and Lysaght, J. (2014). Targeting T Cell Immunometabolism for Cancer Immunotherapy; Understanding the Impact of the Tumor Microenvironment. Front. Oncol. *4*, 107.

Papah, M.B., Brannick, E.M., Schmidt, C.J., and Abasht, B. (2018). Gene expression profiling of the early pathogenesis of wooden breast disease in commercial broiler chickens using RNA-sequencing. PLoS One *13*.

Quiroz-Castañeda, R.E., and Dantán-González, E. (2015). Control of avian coccidiosis: future and present natural alternatives. BioMed Res. Int. *2015*.

Shanmugasundaram, R., Kogut, M.H., Arsenault, R.J., Swaggerty, C.L., Cole, K., Reddish, J.M., and Selvaraj, R.K. (2015). Effect of Salmonella infection on cecal tonsil regulatory T cell properties in chickens. Poult. Sci. pev161.

Stern, C.D. (2005). The chick: a great model system becomes even greater. Dev. Cell *8*, 9–17.

Taylor Jr, R.L., and McCorkle Jr, F.M. (2009). A landmark contribution to poultry science—Immunological function of the bursa of Fabricius. Poult. Sci. *88*, 816–823.

Wang, X., Peebles, E.D., Kiess, A.S., Wamsley, K.G., and Zhai, W. (2019). Effects of coccidial vaccination and dietary antimicrobial alternatives on the growth performance, internal organ development, and intestinal morphology of Eimeria-challenged male broilers. Poult. Sci. *98*, 2054–2065.

Weiss, R.A. (1998). The oncologist's debt to the chicken. Avian Pathol. *27*, S8–S15.

Williams, H.H. (1998). Delmarva's Chicken Industry: 75 Years of Progress. Delmarva Poultry Industry, Inc.

National Chicken Council. "U.S. Broiler Performance". https://www.nationalchickencouncil.org/about-the-industry/statistics/u-s-broiler-performance/. Accessed February 20, 2020.

Effects of Butyrate Supplementation on Energy Metabolism and Inflammation of Dairy Cows During the Calving Transition Period

Effets de la supplémentation en butyrate sur le métabolisme énergétique et le processus inflammatoire chez les vaches laitières durant la période de transition après vêlage

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Abstract

Inflammation is common in dairy cows during the calving transition period. Absorption of gut-derived endotoxins induce inflammation, reducing energy availabile for milk production. Research in other species has shown that butyrate may reduce inflammation by improving gut barrier function, and reducing endotoxin absorption. We evaluated the effects of dietary butyrate supplementation on performance, plasma metabolites, and serum inflammation indicators in dairy cows during the calving transition period. Thirty-seven Holstein heifers (n = 17) and cows (n = 20) were blocked by parity and calving date, and randomly assigned to one of iso-energetic diets containing calcium butyrate (BUT; Proformix; Probiotech Inc., Saint Hyacinthe, QC; 1.42% of diet dry matter (DM)) or a control (CONT; 1.04% palm fat and 0.38% calcium carbonate of diet DM). The closeup diet contained 14.9% starch and 42.5% neutral detergent fiber (NDF), and the fresh diet contained 23.9% starch and 32.0% NDF on a DM basis. Diets were fed d 28 ± 3 before the expected calving date to d 24 ± 3 after calving. Data were analyzed using the FIT model of JMP Pro 14.2 (SAS Institute Inc., Cary, NC). Serum haptoglobin concentrations did not differ between BUT and CONT ($P = 0.76$). However, plasma free fatty acid concentration tended to be lower in BUT on d 7 (885 vs. 1048 μEq/L; $P = 0.10$), while plasma β-hydroxybutyric acid concentration was higher in BUT on d 4 (11.3 vs. 8.11 mg/dL; $P < 0.01$). Milk urea nitrogen concentration was also lower in BUT on d 21 (13.2 vs. 15.8 mg/dL; $P < 0.05$). Additionally, postpartum body weight change tended to be greater in BUT (-3.84 vs. -2.61 kg/d; $P = 0.10$). There were no treatment differences observed in milk yield ($P = 0.73$), 3.5% fat-corrected milk yield ($P = 0.49$), milk fat ($P = 0.21$), milk protein ($P = 0.38$), DMI ($P = 0.33$), or postpartum BCS change ($P = 0.41$). The current study does not support that inflammation is reduced by oral butyrate supplementation, however, butyrate may reduce body fat mobilization in transition cows.

Key words: butyrate, inflammation, free fatty acids

Résumé

L'inflammation est fréquente chez les vaches laitières en période de transition après vêlage. L'absorption d'endotoxines d'origine intestinale provoque l'inflammation, réduisant la quantité d'énergie disponible pour la production de lait. Des recherches menées sur d'autres espèces animales ont montré que le butyrate peut réduire l'inflammation en renforçant la barrière intestinale et en réduisant l'absorption d'endotoxines. Nous avons examiné les effets d'une supplémentation alimentaire en butyrate sur les performances, les métabolites plasmatiques et les indicateurs d'inflammation sérique chez les vaches laitières pendant la période de transition après vêlage. Trente-sept génisses (n = 17) et vaches (n = 20) holstein ont été groupées par parité et date de vêlage, puis assignées au hasard à une des rations iso-énergétiques contenant du butyrate de calcium (BUT; Proformix; Probiotech Inc., Saint-Hyacinthe, QC; 1,42 % de la matière sèche [MS] de la ration) ou à une ration témoin (CONT; 1,04 % d'huile de palme et 0,38 % de carbonate de calcium sur une base de MS de la ration). La ration de préparation au vêlage contenait 14,9 % d'amidon et 42,5 % de fibres au détergent neutre (NDF); la ration d'après vêlage contenait 23,9 % d'amidon et 32,0 % de NDF sur une base de MS. Les rations ont été servies de 28 ± 3 jours avant la date prévue de vêlage jusqu'à 24 ± 3 jours après le vêlage. Les données ont été analysées à l'aide du modèle FIT de JMP Pro 14.2 (SAS Institute Inc., Cary, Caroline du Nord). Les concentrations sériques d'haptoglobine n'ont pas différé entre les traitements BUT et CONT (P = 0,76). Cependant, la concentration plasmatique en acides gras libres a eu tendance à être plus faible dans le traitement BUT à j7 (885 vs. 1048 µÉq/L; P = 0,10), tandis que la concentration plasmatique en acide β-hydroxybutyrique était plus élevée dans le traitement BUT au j4 (11,3 vs 8,11 mg/dL; P < 0,01). La concentration d'azote uréique dans le lait était également plus faible dans le traitement BUT à j21 (13,2 vs 15,8 mg/dL; P < 0,05). En outre, la variation de poids corporel après vêlage a eu tendance à être plus importante dans le traitement BUT (-3,84 vs -2,61 kg/j; P = 0,10). Aucune différence de traitement n'a été observée pour le rendement laitier (P = 0,73), le rendement en lait corrigé à 3,5 % de matière grasse (P = 0,49), la matière grasse du lait (P = 0,21), les protéines du lait (P = 0,38), la CVMS (P = 0,33) ou la variation d'état corporel post-partum (P = 0,41). L'étude actuelle ne permet pas d'affirmer que l'inflammation est réduite par une supplémentation alimentaire en butyrate; cependant, le butyrate peut freiner la mobilisation des graisses corporelles chez les vaches en transition.

Introduction

Dairy cows are provided high allocations of grain following calving to meet high energy demands for milk production. However, increased grain consumption increases the flow of fermentable carbohydrates to the hindgut, increasing hindgut fermentation (Abdela, 2016). If excessive fermentation occurs, high osmotic pressure and a drop in pH induce inflammation and damage hindgut epithelial cells, thus increasing gut permeability and allowing absorption of bacterial endotoxins (Gressley et al., 2011; Tao et al., 2014; Ye et al., 2016). Systemic inflammation may then occur, activating the immune system and further increasing the cow's energy requirements (Turner, 2009). To compensate for energy demands from lactation and inflammation, cows mobilize free fatty acids (FFA) from adipose tissue which are transported to the liver where they can be completely oxidized to ATP, or incompletely oxidized to produce

ketones if insufficient glucogenic fuels are present (Roche et al., 2009). Some fat mobilization and ketone production is normal, however, excess fat mobilization can cause excessive ketone production and triglyceride storage in the liver, further decreasing DMI and potentially exacerbating negative energy balance (Zarrin et al., 2017).

Butyrate supplementation may be an option to mitigate acute gut inflammation through suppressing protein and gene expression responsible for activating inflammatory responses in colonocytes (Yin et al., 2001). Butyrate may also abate epithelial cell damage following excessive fermentation as butyrate provides energy to epithelial cells (Chen et al., 2018). Further, butyrate increases epithelial cell proliferation (Guilloteau et al., 2010) and modulates tight junction assembly (Peng et al., 2009), and as such, improves gut barrier function in other species (Dong et al., 2016; Chen et al., 2018; Elnesr et al., 2020). Therefore, butyrate supplementation may reduce LPS absorption, decreasing systemic inflammation and the associated energy requirement, ultimately reducing fat mobilization and improving energy status. As less energy is being allocated towards the immune system due to reduced inflammation, milk yield may increase (Bradford et al., 2015). However, the efficacy of butyrate supplementation for alleviating inflammation has not been extensively evaluated in transition dairy cows. The objective of the present study was to assess the effects of butyrate supplementation during the calving transition period on DMI, blood parameters, postpartum milk production, and inflammation.

Methods

This study was conducted from August to December, 2019, at the University of Alberta's Dairy Research and Technology Centre. Thirty-seven Holstein heifers (n = 17) and cows (n = 20) were blocked by parity and expected calving date, and randomly assigned to one of iso-energetic diets containing calcium butyrate (BUT; Proformix; Probiotech Inc., Saint Hyacinthe, QC; 1.42% of diet dry matter (DM)) or a control (CONT; 1.04% palm fat and 0.38% calcium carbonate of diet DM). The closeup diet contained 14.9% starch and 42.5% neutral detergent fiber (NDF), and the fresh diet contained 23.9% starch and 32.0% NDF on a DM basis. Diets were fed d 28 ± 3 before the expected calving date to d 24 ± 3 after calving. Animals were housed in tie-stalls with individual feed bunks for the duration of the study. Milk yield and DMI were recorded daily, and milk composition was analyzed on d 7 ± 3, 14 ± 3, and 21 ± 3. Plasma was collected on d -28 ± 3, -4 ± 1, 1, 4, 7, 21 ± 3 relative to calving, and serum was collected on d -4 ± 1, 1, 4, and 7 relative to calving. Plasma was analyzed for FFA and β-Hydroxybutyrate (BHB), and serum was analyzed for haptoglobin (Hp). Body condition score (BCS) and body weight (BW) were recorded on d 1 and 24 ± 3 and post-calving changes were calculated. Data were analyzed using the FIT model procedure of JMP Pro 14.2 (SAS Institute Inc., Cary, NC).

Results

Serum haptoglobin concentrations did not differ between BUT and CONT ($P = 0.76$). However, in BUT plasma BHB was higher on d 4 (Table 1), plasma FFA tended to be lower on d 7, and MUN was lower on d 21. Postpartum BW change also tended to be greater in BUT (-3.84 vs. -

2.61 kg/d; $P = 0.10$). There were no treatment differences observed in milk yield ($P = 0.73$), 3.5% fat-corrected milk yield ($P = 0.49$), milk fat ($P = 0.21$), milk protein ($P = 0.38$), DMI ($P = 0.33$), or postpartum BCS change ($P = 0.41$).

Table 1. Effects of butyrate supplementation on concentration of milk urea nitrogen (MUN), serum haptoglobin (Hp), plasma free fatty acids (FFA), and plasma beta-hyroxybutyrate (BHB).

| | Treatment | | | |
	Control	Butyrate	SE	*P*-value
Plasma BHB, mg/dL				
1 d	8.74	9.52	0.53	0.46
4 d	8.11	11.3	0.55	< 0.01
7 d	8.83	10.2	0.53	0.19
Plasma FFA, µEq/L				
1 d	602	606	49	0.96
4 d	868	764	50	0.30
7 d	1048	885	50	0.10
MUN, mg/dL				
7 d	12.3	12.3	0.52	0.98
14 d	13.5	12.4	0.54	0.27
21 d	15.8	13.2	0.54	< 0.05

Conclusion

Butyrate supplementation did not affect milk yield or components, DMI, or postpartum changes BCS. In addition, the present study does not support that inflammation is reduced by butyrate supplementation, however, butyrate supplementation may reduce body fat mobilization and MUN, indicating modifications in energy and protein metabolism in transition cows.

Acknowledgments

References

Abdela, N. 2016. Sub-acute Ruminal Acidosis (SARA) and its Consequence in Dairy Cattle: A Review of Past and Recent Research at Global Prospective. Achiev. Life Sci. 10:187–196.

Bradford, B. J., K. Yuan, J. K. Farney, L. K. Mamedova, A. J. Carpenter. 2015. Invited review: Inflammation during the transition to lactation: New adventures with an old flame. J. Dairy Sci. 98:6631–6650.

Chen, G., X. Ran, B. Li, Y. Li, D. He, B. Huang, S. Fu, J. Liu, and W. Wang. 2018. Sodium Butyrate Inhibits Inflammation and Maintains Epithelium Barrier Integrity in a TNBS-induced Inflammatory Bowel Disease Mice Model. EBioMedicine 30:317–325.

Dong, L., X. Zhong, J. He, L. Zhang, K. Bai, W. Xu, T. Wang, and X. Huang. 2016. Supplementation of tributyrin improves the growth and intestinal digestive and barrier functions in intrauterine growth-restricted piglets. Clin Nutr. 35:399–407.

Elnesr, S. S., M. Alagawany, H. A. Elwan, M. A. Fathi, and M. R. Farag. 2020. Effect of sodium butyrate on intestinal health of poultry – A Review. Ann. Anim. Sci. 20:29–41.

Gressley, T.F., M.B. Hall, and L.E. Armentano. 2011. Ruminant nutrition symposium: Productivity, digestion, and health responses to hindgut acidosis in ruminants. J. Anim. Sci. 89:1120–1130

Guilloteau, P., L. Martin, V. Eeckhaut, R. Ducatelle, R. Zabielski and F. Van Immerseel. 2010. From the gut to the peripheral tissues: the multiple effects of butyrate. Nutrition Research Reviews 23:366–384.

Peng, L., Z. R. Li, R. S. Green, I. R. Holzman, and J. Lin. 2009. Butyrate enhances the intestinal barrier by facilitating tight junction assembly via activation of AMP-activated protein kinase in Caco-2 cell monolayers J. Nutr. 139:1619–1625.

Roche, J. R., N. C. Friggens, J. K. Kay, M. W. Fisher, K. J. Stafford, and D. P. Berry. 2009. Invited review: Body condition score and its association with dairy cow productivity, health, and welfare. J. Dairy Sci. 92:5769–5801.

Tao, S., Y. Duanmu, H. Dong, Y. Ni, J. Chen, X. Shen, and R. Zhao. 2014. High concentrate diet induced mucosal injuries by enhancing epithelial apoptosis and inflammatory response in the hindgut of goats. PLoS One 9:e111596.

Turner, J. 2009. Intestinal mucosal barrier function in health and disease. Nat. Rev. Immunol. 9:799–809.

Ye, H., J. Liu, P. Feng, W. Zhu, and S. Mao. 2016. Grain-rich diets altered the colonic fermentation and mucosa-associated bacterial communities and induced mucosal injuries in goats. Sci. Rep. 6:20329.

Yin, L., G. Laevsky, and C. Giardina. 2001. Butyrate Suppression of Colonocyte NF- κB Activation and Cellular Proteasome Activity. J. Bio. Chem. 276:44641–44646.

Zarrin, M., L. Grossen-Rösti, R. M. Bruckmaier, and J. J. Gross. 2017. Elevation of blood β-hydroxybutyrate concentration affects glucose metabolism in dairy cows before and after parturition. J. Dairy. Sci. 100:232–2333.

Effects of Lipid Inclusion and Saturation of Dietary Fatty Acids on Nutrient Transport Across the Ruminant Gastrointestinal Tract

Effets de l'inclusion de lipides et de la saturation des acides gras alimentaires sur le transport des nutriments dans le tractus gastro-intestinal des ruminants

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Abstract

The objective of this study is to evaluate the effects that supplementing cattle with C18 fatty acids of differing saturation can have on rumen tissue fatty acid composition and reticulo-rumen short chain fatty acid absorption. Eight heifers were used in a duplicate 4 × 4 Latin Square Design. Heifers were supplemented with fatty acids at a fixed rate of 0.06% of BW. Treatments included control (CON; no supplemental fatty acid), saturated (SAT; supplemental stearic acid, 70% C18:0), unsaturated (UNSAT; supplemental linoleic acid, 74% C18:2, 36% conjugated linoleic acid), and control isocaloric treatment (CON-ISO; no supplemental fatty acid, but isocaloric to both SAT and UNSAT). Total-tract permeability was measured using Cr-EDTA and short chain fatty acid absorption across the reticulo-rumen was measured by the temporarily isolated and washed reticulo-rumen technique. Rumen tissue biopsies and blood samples were collected at the beginning and end of each period. Rumen tissue will be measured for fatty acid composition and blood will be analyzed for fatty acid composition, non-esterified fatty acids, beta-hydroxy butyrate, and cholesterol.

Résumé

L'objectif de cette étude est d'évaluer les effets possibles de la supplémentation en acides gras C18 de divers degrés de saturation sur la composition en acides gras des tissus du rumen et sur l'absorption des acides gras à chaîne courte par le réticulo-rumen chez les bovins. Huit génisses ont été réparties selon un dispositif en carré latin double 4 × 4. Les génisses ont reçu un supplément d'acides gras à dose fixe de 0,06 % du poids corporel. Les traitements comprenaient un traitement témoin (CON; pas d'acide gras supplémentaire), un traitement saturé (SAT; acide stéarique supplémentaire, 70 % C18:0), un traitement insaturé (UNSAT; acide linoléique supplémentaire, 74 % C18:2, 36 % d'acide linoléique conjugué) et un traitement isocalorique témoin (CON-ISO; pas d'acide gras supplémentaire, mais isocalorique à la fois à SAT et à UNSAT). La perméabilité totale du tube digestif a été mesurée à l'aide de Cr-EDTA et l'absorption des acides gras à chaîne courte dans le réticulo-rumen a été mesurée par la technique du réticulo-rumen lavé et temporairement isolé. Des biopsies des tissus du rumen et des échantillons de sang ont été prélevés au début et à la fin de chaque période. La composition en acides gras des tissus du rumen sera mesurée, et le sang sera analysé pour sa composition en acides gras, en acides gras non estérifiés, en bêta-hydroxybutyrate et en cholestérol.

Introduction

Supplemental lipids are commonly included in diets for ruminants (Doreau and Ferlay 1994). These lipids are components of feedstuffs or through the addition of specific lipid additives (Loften et al. 2014). Most commonly, these lipid additives are used to increase the energy density of the diet (Hess et al. 2008), but can also be used to modulate the fatty acid (**FA**) composition of body tissues or induce metabolic changes. Verdugo (2016) reported changes in rumen tissue FA composition as well as short-chain fatty acid (**SCFA**) absorption when supplementing Holstein steers fed diets with differing supplements (canola vs. flax oil) and for diets with differing lipid content. In that study, steers supplemented with more unsaturated FA (flax) tended to have higher proportion of unsaturated FA (C18:2) in rumen tissue but had lesser rates of propionate uptake via passive diffusion and total uptake of butyrate. Additionally, steers supplemented with saturated FA tended to have greater butyrate uptake via passive diffusion. While that study showed that FA concentration and saturation can affect function of the gastrointestinal tract, it is not clear which FA may induce an effect. The hypothesis is that ruminal tissue FA composition will mirror that of dietary supply, and dietary unsaturated FA supplements high in conjugated linoleic acid will increase permeability and decrease SCFA absorption as compared to FA supplements high in unsaturated C18:0. The objective is to characterize the effects that FA supplements of differing C18 FA isomers will have on ruminal tissue FA composition, total tract barrier function and absorption of SCFA across the reticulo-rumen.

Materials and Methods

Eight cannulated Hereford crossbred heifers were blocked by weight and organized in a duplicate 4 × 4 Latin square design. The sequence of treatments was balanced for carry-over effects. Heifers were assigned to one of four treatments: **CON** (no supplemental FA), **SAT** (supplemental stearic,

70% C18:0; Industrene 7018, PMC Biogenix, Memphis TN, USA), **UNSAT** (supplemental linoleic, 74% C18:2, 38% CLA; Pamolyn 300, Eastman, Kingsport TN, USA), or **CON-ISO** (no supplemental FA with diets formulated to be isocaloric to both FA diets). Experimental periods consisted of 28 d in duration. Day 1 to 7 was a washout period with diets formulated to be slightly deficient in energy (NEg = 0.73 Mcal/kg) to promote lipid reserve mobilization and limit carry-over effects from prior periods. From d 8 to 28, heifers were fed their respective treatment diets (Table 1) at a fixed rate (2.25% BW on DM basis). Experimental diets were adjusted to have a DM of 75% using water to reduce the risk for feed sorting.

Heifers were supplemented with FA from d 8 to 28 at a fixed rate of 0.06% body weight, equating to 2.73% of dietary DMI. SAT heifers received their dose directly into the rumen while UNSAT heifers were abomasally infused to avoid biohydrogenation. CON and CON-ISO heifers received a sham treatment by briefly locating the reticulo-omasal orifice. Heifers were infused three times daily (0900, 1200, and 1500 h) with each dose being 33.3% of total daily allocation.

Table 1. Ingredient and predicted chemical composition of diets for heifers supplemented with no fatty acid (CON), 2% DMI of C18:0 (SAT), 2% DMI of C18:2 (UNSAT), or no fatty acid but isocaloric to lipid treatments (CON-ISO).

Inclusion, % DM	Washout	CON	CON-ISO	SAT	UNSAT
Grass hay	79.02	-	-	-	-
Canola meal pellet	11.24	-	-	-	-
Alfalfa hay	-	44.94	44.94	44.94	44.94
Oat hulls	-	10.69	3.93	10.69	10.69
Dry rolled barley	-	34.49	41.37	31.65	31.65
Mineral vitamin	9.74	9.74	9.74	9.74	9.74
Urea	-	0.13	-	0.24	0.24
Supplemental fatty acid	-	-	-	2.73	2.73
Predicted chemical composition					
aNDFom, % DM	50.29	29.96	26.48	29.41	29.41
Starch, % DM	6.00	27.35	30.69	25.65	25.65
EE, % DM	2.45	2.36	2.35	5.01	5.01
C18:0 added (g/d)	-	-	-	201.23	0.29
C18:2 added (g/d)	-	-	-	0.00	212.73

On d 8 and 28 of each period, rumen tissue biopsies were collected at 0700 h according to Steele et al. (2009). Venous blood samples were collected at 0800 h. Rumen tissue samples and plasma will be analyzed for FA composition by gas chromatography (Surkhija and Palmquist 1988). Blood non-esterified fatty acids, beta-hydroxybutyrate, and cholesterol will be measured using commercial kits. Total tract permeability will be evaluated according to Zhang et al. (2013) from d 25 to 27. Urine Cr concentration will be measured as described by (Vicente et al. 2004). On d 28, absorption of SCFA was measured by the temporarily isolated and washed reticulo-rumen method (Care et al. 1984) with buffers described by Zhang et al. (2013). Buffer was sampled at 0, 5, and 45 min. Samples will be analyzed for Cr (Vicente et al. 2004) and SCFA (Khorasani et al. 1996) concentrations to enable calculation of SCFA disappearance.

Data will be analyzed as a Latin square design using the mixed model of SAS (version 9.4, Cary, NC, USA). Treatment, square, and period will be considered as fixed effects with the randomized effect of heifer. Means will be separated using Tukeys means separation test.

References

Care, A.D., Brown, R.C., Farrar, A.R., and Pickard, D.W. 1984. Magnesium absorption from the digestive tract of sheep. Q. J. Exp. Physiol.: 577–587.

Doreau, M., and Ferlay, A. 1994. Digestion and utilisation of fatty acids by ruminants. Anim. Feed Sci. Technol. **45**: 379–396. doi:10.1016/0377-8401(94)90039-6.

Hess, B.W., Moss, G.E., and Rule, D.C. 2008. A decade of developments in the area of fat supplementation research with beef cattle and sheep. J. Anim. Sci. **86**: 188–204. doi:10.2527/jas.2007-0546.

Khorasani, G.R., Okine, E.K., and Kennelly, J.J. 1996. Forage Source Alters Nutrient Supply to the Intestine Without Influencing Milk Yield. J. Dairy Sci. **79**: 862–872. Elsevier. doi:10.3168/jds.S0022-0302(96)76435-4.

Loften, J.R., Linn, J.G., Drackley, J.K., Jenkins, T.C., Soderholm, C.G., and Kertz, A.F. 2014. Invited review: Palmitic and stearic acid metabolism in lactating dairy cows. J. Dairy Sci. **97**: 4661–4674. Elsevier. doi:10.3168/jds.2014-7919.

Steele, M.A., AlZahal, O., Hook, S.E., Croom, J., and McBride, B.W. 2009. Ruminal acidosis and the rapid onset of ruminal parakeratosis in a mature dairy cow: a case report. Acta Vet. Scand. **51**: 39. doi:10.1186/1751-0147-51-39.

Surkhija, P.S., and Palmquist, D.L. 1988. Rapid method of determination of total FA content and composition of feedstuffs and faeces. J. Agric. Food Chem. **36**: 1202–1206.

Verdugo, A. 2016. Effect of lipid supplementation on ruminal epithelial membrane fatty acid composition and short-chain fatty acid absorption. University of Saskatchewan.

Vicente, F., Sarraseca, A., De Vega, A., and Guada, J.A. 2004. Performance of several Cr and Yb analytical techniques applied to samples of different biological origin (digesta or faeces). J. Sci. Food Agric. **84**: 2035–2040. doi:10.1002/jsfa.1908.

Zhang, S., Albornoz, R.I., Aschenbach, J.R., Barreda, D.R., and Penner, G.B. 2013. Short-term feed restriction impairs the absorptive function of the reticulo-rumen and total tract barrier function in beef cattle 1. J. Anim. Sci.: 1685–1695. doi:10.2527/jas2012-5669.

Inherent digestive tract insufficiency in monogastric animals: culpability of the gut microbiome and dietary approaches for optimizing intestinal health

Insuffisance digestive inhérente chez les animaux monogastriques : culpabilité du microbiome intestinal et approches alimentaires pour optimiser la santé intestinale

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Abstract

Although pigs and poultry are highly efficient in converting feed to food products, they still excrete significant amounts of undigested nutrients. For example, broilers lose almost 25-30% of ingested dry matter, 20-25% of gross energy, 30-50% of nitrogen and 45-55% of phosphorus intake in the manure. Undigested nutrients have negative implications on production efficiency, profitability and sustainability of farm operations. Moreover, the undigested nutrients serve as substrates for enteric pathogens with implications to animal (e.g. *E. coli*, *clostridia*) health and human (e.g. *salmonella*, *campylobacter*) health via contained animal products. The peculiarity is that feedstuffs contain anti-nutritional factors (ANF) such as phytic acid or fractions that are not degraded sufficiently or indeed at all by the conditions and the array of digestive enzymes in the gastrointestinal tract. Moreover, the newly hatched chicks and weaned piglets have limited capacity to express endogenous enzymes. Arguably with restriction on use of preventive antibiotics, there is need to explore and implement alternative dietary approaches to enhance digestive function. Supplemental feed enzymes improve nutrient utilization linked to reduced nutrients excretion and enteric pathogen load. Moreover, nutritional strategies for stimulating juvenile gut maturation (e.g. epidermal growth factor, feed structure), nurturing favorable gut microflora (e.g. probiotics, prebiotic), suppressing enteric pathogens (e.g. organic acids, low crude protein diets) and maintaining intestinal integrity (e.g. yeast metabolites) should be integral in AGP free feeding programs. These dietary approaches in combination with improvements in management, vaccination programs and/or genetics will be effective in optimizing growth performance of poultry and pigs raised without or with reduced antibiotics for growth promotion.

Résumé

À la lumière des progrès de l'amélioration génétique et des restrictions sur l'utilisation des antibiotiques stimulateurs de croissance, le dysfonctionnement du tractus gastro-intestinal est considéré comme un facteur potentiel limitant la survie et la productivité des animaux de ferme monogastriques élevés en régie intensive. Cet article donnera un aperçu des avancées récentes sur les technologies d'alimentation animale susceptibles d'optimiser la santé et le fonctionnement des

intestins et d'améliorer l'utilisation des aliments. Les porcs et les volailles convertissent très efficacement les rations animales en produits pour la consommation humaine. Cependant, ils excrètent malgré tout des quantités importantes de nutriments non digérés, ce qui a des conséquences négatives sur l'efficacité de la production, la rentabilité des exploitations agricoles et la durabilité de ce secteur d'activité. En outre, les nutriments non digérés servent de substrats aux agents pathogènes entériques. La particularité des aliments pour animaux est qu'ils contiennent des facteurs antinutritionnels (FAN), tels que l'acide phytique, ou des fractions qui sont insuffisamment ou nullement dégradées par les enzymes digestives endogènes. En réduisant l'excrétion de nutriments et la charge entérique pathogène, l'ajout aux rations animales d'enzymes alimentaires ciblant divers FAN peut améliorer l'utilisation des nutriments, la rentabilité et la durabilité. Les stratégies de production sans antibiotiques stimulateurs de croissance entraîneront une incidence plus élevée de problèmes de santé intestinale et allongeront les périodes de vulnérabilité immunitaire. Ainsi, les stratégies nutritionnelles visant à supprimer les agents pathogènes entériques (par exemple, les acides organiques, les régimes à faible teneur en protéines brutes) et à maintenir l'intégrité intestinale (par exemple, les probiotiques, les métabolites de levure, le facteur de croissance épidermique) feront partie intégrante des programmes d'alimentation sans antibiotiques stimulateurs de croissance. Ces approches alimentaires, combinées à des améliorations aux pratiques de gestion, aux programmes de vaccination ou à la génétique, seront efficaces pour optimiser les performances de croissance des volailles et des porcs produits sans ou avec une quantité réduite d'antibiotiques pour favoriser la croissance.

Introduction

Advances in genetics has certainly produced commercial strains of poultry and pig with greater performance (e.g. growth, reproduction) with minimal feed input. For example, over the last 5 decades, the body weight of broilers at 42 days has increased by 25-50 g per year and the feed conversion ratio to 2 kg body weight has improved 2-3 points annually (Havenstein et al., 2003; Gous, 2010; Aviagen, 2019). With the introduction of crosses in the early 60's, specialization in dam and sire lines have been very successful in effecting genetic improvement of economically important traits in pigs, especially daily gain, backfat thickness, feed efficiency and litter size. An annual genetic progress for gain of +20 g/day, lean meat of +0.5% and litter size of +0.2 piglet/litter has been achieved over the last few decades (Krupa et al., 2017; Merks, 2018). The nutrition of these animals has also evolved overtime but not as much as genetic advances(Kiarie and Mills, 2019); for example genetic selection brought about by breeding companies is responsible for 85-90% of the improvements in broiler growth, and advances in nutritional management contributed only 10-15% (Havenstein et al., 2003). However, the necessity to achieve and sustain genetic potential has been the driving force behind continuous advances in nutrition concepts seen in modern day commercial pig and poultry enterprises. In this context, feeding, a major control point of profitability has evolved and progressed both in terms of understanding digestive physiology and metabolism, and in the more precise evaluation of the quality of dietary raw materials. Advances in monogastric nutrition is clearly exemplified by the widespread adoption of net energy, standardized ileal digestible amino acids ideal ratio and digestible phosphorous concepts enabling nutritionists to formulate cost-effective and optimal diets (NRC, 1994, 2012). However, the modern-day nutritionists perceive dysfunctional gastrointestinal tract as a potential rate-limiting factor in the survival and productivity of monogastric farm animals (Kiarie et al., 2019a).

This perception has been fostered by the emergence of ideas and concepts concerning the development and function of the digestive tract in the light of advances in genetic improvement and restriction on the use of antibiotic growth promoters and anti-coccidial drugs. The intention of this review is to provide insights in digestive tract insufficiency in monogastric farm animals, the implications of gut microbiome and dietary approaches for enhancing gut function and health without recourse to antimicrobial growth promoters.

What is it about the digestive tract in monogastric farm animals?

Undoubtedly, pigs and poultry are highly efficient in converting feed to food products, but they still excrete significant amounts of undigested nutrients. For example, broilers lose almost 25-30% of ingested dry matter, 20-25% of gross energy, 30 -50% of nitrogen and 45-55% of phosphorus intake in the manure (Ravindran, 2012). Pigs of different breeds and ages were observed to digest 78% of gross energy in typical corn and soybean meal diet (Urriola and Stein, 2012). Addition of 30% corn dried distiller's grains with solubles to this diet resulted in further reduction of digestible gross energy.

I. Juvenile gut biology

Genetic selection for increased meat production efficiency has dramatically altered the physiological timeline of broiler chickens (Ferket, 2012). For example, between 2005 and 2010, duration to raise 2.5 kg broiler reduced by 0.74 days per year (Gous, 2010). The significance of decreasing time to slaughter weight is that embryonic and early post-hatch periods constitute a greater proportion of a bird's life. For example, 21-day incubation period and the 10-day post-hatch period of the chick accounts for about 50% of a 2.5 kg broiler; yet gut maturation does not happen until after 10 days post-hatch (Uni and Ferket, 2004). Thus, the capacity for the intestine to absorb and assimilate nutrients may pose a proximal constraint upon the rate of growth of newly hatched chicks. In pigs, the gastrointestinal tract (GIT) of the newly weaned pig is poorly developed having: (1) an immature immune system, (2) limited enzyme secretory capacity, (3) sensitive to allergenic feed proteins, and (4) unstable gut microbiota (Lindemann et al., 1986; Pluske, 2016).

II. Inherent digestive insufficiency

Feedstuffs contains anti-nutritional factors (ANF) such as phytic acid or fractions that are not degraded sufficiently or indeed at all by the conditions and the array of digestive enzymes in the gastrointestinal tract (Kiarie et al., 2013; Kiarie et al., 2016; Kiarie and Mills, 2019). This inherent digestive inefficiency in monogastric animals is seen as the reason of commercial development and application of exogenous feed enzymes technology. Indigestible complexes can impede normal digestion and absorption processes of nutrients including carbohydrates and protein (Slominski, 2011). For complete monogastric diets, the digestibility of dry matter varies between 70 and 90% but the variation is larger for specific feed ingredients (10 to 100%). Most of the variation in dry matter digestibility is related to the presence of dietary fiber (DF) which is less

digestible than other nutrients (<40% *vs.* 80-100% for starch, sugars, fat or protein) and reduces the digestibility of other dietary nutrients such as crude protein and fat (NRC, 1994, 2012). The consequences of variable and low nutrient digestibility range from economic through increased feed costs, proliferation of pathogens in the gut, poor feed efficiency to ecological through nutrient loading and emissions into the environment (Kiarie et al., 2013; Kiarie et al., 2016; Kiarie et al., 2019b). Moreover, undigested feed increases visceral weight, consequently increasing utilization of dietary energy and amino acids for maintenance at the expense of tissue deposition (growth) (Cant et al., 1996; Agyekum et al., 2012).

Culpability of gut microbiota in the context of digestive insufficiency

The GIT host a diverse community of organisms with symbiotic relationship with the host. The balance in this ecosystem is of crucial importance in maintaining nutritional, physiological and immunological functions of the host. The diversity of bacterial populations within a micro-habitat in the GIT is influenced by factors such as digesta flow rate, pH, anoxic conditions, types of endogenous and dietary substrates, inhibitory factors such as bacteriocins and short chain fatty acids (SCFA), and competitive advantage (Kiarie et al., 2013). Bacteria in the gut derive most of their carbon, nitrogen and energy from luminal compounds (dietary and/or endogenous) which are either resistant to attack by digestive fluids or absorbed so slowly by the host that bacteria can successfully compete for them. Thus, undigested nutrients in the gut promotes blooming of bacteria in the ceca and in the environment via excretion. It is likely that certain nutrients and their associated physico-chemical effects play a major role in maintaining the balance of the microflora in specific micro-habitats, and subsequently in determining whether a pathogenic bacterium proliferates. For example, in poultry there is greater risk of an outbreak of necrotic enteritis (NE) with the use of viscous grains (barley, wheat and rye) (Timbermont et al., 2011). This has been associated with high digesta viscosity, decreased nutrient digestibility and prolonged intestinal transit time, thus favouring growth of *Clostridium perfringens* in the upper gut (Kaldhusdal and Skjerve, 1996; Timbermont et al., 2011). In swine, viscous fibres have also been linked to exacerbation of post-weaning collibacillosis and swine dysentery (SD) (Pluske et al., 2002). The detrimental effects of soluble fibres in swine have been associated with the increasing digesta viscosity, undigested nutrients in the GIT and endogenous secretions. Furthermore, an increased flow of ileal undigestible protein in the hindgut can result in proteolytic fermentation that can negatively affect performance and health. Arguably, the use of AGP in the past markedly reduced negative consequences of feeding such feedstuffs and as a result performance was maintained on diets that otherwise would be problematic. Indeed, earlier studies demonstrated that the growth-depressing effect of feeding broiler chickens feedstuffs that depressed nutrient digestibility was ameliorated by sub-therapeutic antibiotic supplementation (Marquardt et al., 1979; Antoniou and Marquardt, 1982). Furthermore, (Smulders, 1999) showed that sub-therapeutic antibiotics were more effective in diets with low digestible protein content *versus* in diets with high digestible protein content.

Dietary concepts and approaches for maintaining functional gut without recourse to antimicrobial growth promoters

I. Stimulating functional gastrointestinal (GIT) development
There are numerous functional ingredients, factors and /or nutrients that are known to enhance GIT development and could be strategically applied in starter diets to enhance digestive capacity and resilience to enteric pathogens. For example, epidermal growth factors (Kim et al., 2017), yeast metabolites (Kiarie et al., 2010; Kiarie et al., 2011; Kiarie et al., 2012; Leung et al., 2019) and organic acids(Kiarie et al., 2018). Poultry requires a certain amount of diet structure for proper gut development and functionality. For example, data suggest course feed structure (created by grain particle size manipulation, inclusion of insoluble fiber or access to litter) may improve digestive function and development including increased secretion of acid and enzymes (Mateos et al., 2012). Diet structure plays an important role in stimulating gizzard development, controlling digesta passage rate and improving gut motility by enhancing endocrine cholecystokinin release which stimulates the secretion of pancreatic enzymes and gastroduodenal refluxes (Mateos et al., 2012). This was demonstrated by (Xu et al., 2015) who replaced (wt/wt) finely ground corn (294 µm, as per the industry standards) with 25% and 50% course ground corn (1,362 µm) to create three diets with mean particle sizes of 432, 541 and 640 µm. These diets were fed to broiler chickens to 50 days of age to conclude that birds fed diets containing 25 and 50% course corn exhibited increased BW, improved FCR, and increased apparent ileal digestibility of energy and crude protein, linked due to enhanced gizzard development. A longer retention time in the gizzard leads to more exposure of feed particles to gastric juices that improves digestion, thereby contributing to a better feed efficiency.

II. Reducing undigested substrates by use of feed enzymes
Beneficial effects of feed enzymes are inextricably linked to the amount of the undigested fat, protein and starch in the ileum. Accelerated intestinal digestion and removal of what would otherwise be apparently undigested without feed enzyme must clearly limit the nutrients available for the microbes (Kiarie et al., 2013; Munyaka et al., 2016). As illustrated in Table 1, almost all commercial feed enzymes address such ANF to varying degrees (Kiarie et al., 2016). Therefore, the utility of feed enzyme technology in monogastric nutrition is to degrade ANF in feedstuffs and to complement endogenous enzymes in gut compromised animals particularly hatchlings and weanlings. As alluded to microbiome profile and metabolic function is partly reflective of feed composition (Apajalahti et al., 2004, 2007). It is therefore plausible that manipulating diet digestibility will influence GIT microbiome (Kiarie et al., 2013).

Table 1. Commercial feed enzymes and target substrates

Target feedstuff	Target substrate (anti-nutritional factor)	Anti-nutritional effect	Enzyme
All plant-derived ingredients	Phytate	↓ Digestible minerals ↑ P and Ca excretion	Phytase
Cereal grains and co-products, fibrous plant materials	Arabinoxylans, β-glucan, cell wall matrix	↑ viscosity and digesta retention time ↓ nutrient absorption ↑ activity of intestinal microbiota ↓ accessibility of nutrients by physical entrapment	Xylanase β – glucanase Mannase Cellulase Pectinase
Cereal grains and grain legumes	Resistant Starch	↓ Energy value of ingredients ↑ substrate for gut microbiota	Amylase
All plant protein sources	Oligosaccharides typsin inhibitors, antigens	↓ Energy and protein value of ingredients ↑ substrate for pathogens	Protease
All plant-derived ingredients	Phytate	↓ Digestible minerals ↑ P and Ca excretion	Phytase

Source: Kiarie et al., 2016.

Furthermore, fiber degrading enzymes could release hydrolysis products "prebiotic" that can modulate intestinal microbiota (Courtin et al., 2008; Kiarie et al., 2013; Wealleans et al., 2017; Bedford, 2018). Utility of feed enzymes in transitioning weaned pigs has also been demonstrated in studies that compared feed enzymes and antimicrobial growth promoters. For example, a supplemental multi-enzyme blend caused similar performance and digestibility of gross energy and crude protein to pigs fed an antibiotic in the early phase of weaning (Table 2) (Kiarie et al., 2015). Supplemental xylanase reduced grow-finishing pig mortality from 4.0 % to 2.4% and improved gain: feed from 0.286 to 0.290 (Figure 1) (Zier-Rush et al., 2016). The aforementioned studies and others in the literature (Bedford and Cowieson, 2012; Kiarie et al., 2013) suggests that feed enzymes could be a tool for not only improving feedstuffs digestibility but could also influence gut health, livability, uniformity and carcass value in pigs.

Table 2. Effects of feeding an antibiotic (PC) and a multi-enzyme (ME) blend on growth performance and coefficients of total tract apparent digestibility (CTTAD) in nursery pigs

Item	Phase 1, days 0-14					Phase 2, days 15-42					
	Performance, g/d			CTTAD		Performance, g/d				CTTAD	
	ADG	ADFI	G:F	GE	CP	BW42	ADG	ADFI	G:F	GE	CP
PC	264^a	327	0.82^a	0.80^a	0.75^a	28.9^a	664^a	989	0.67	0.73^a	0.70^a
NC	217^b	320	0.68^b	0.73^b	0.70^b	27.2^b	626^{ab}	996	0.64	0.70^b	0.66^b
ME	265^a	372	0.71^b	0.78^a	0.74^a	27.7^{ab}	618^b	983	0.63	0.74^a	0.71^a
SEM	15.5	18.4	0.02	1.41	1.85	0.54	14.0	29.6	0.01	0.66	1.25

Diets were: 1) Positive control (PC) + antibiotic (0.5% Mecadox), 2) Negative control (NC, no additives), and 3) NC + ME (ME, 4,000 U of xylanase, 150 U of β-glucanase, 500 U of protease and 1,000 U/kg of amylase per kg of feed). [a,b]Means within a column not having the same superscript are significantly different ($P<0.05$). (Kiarie et al., 2015)
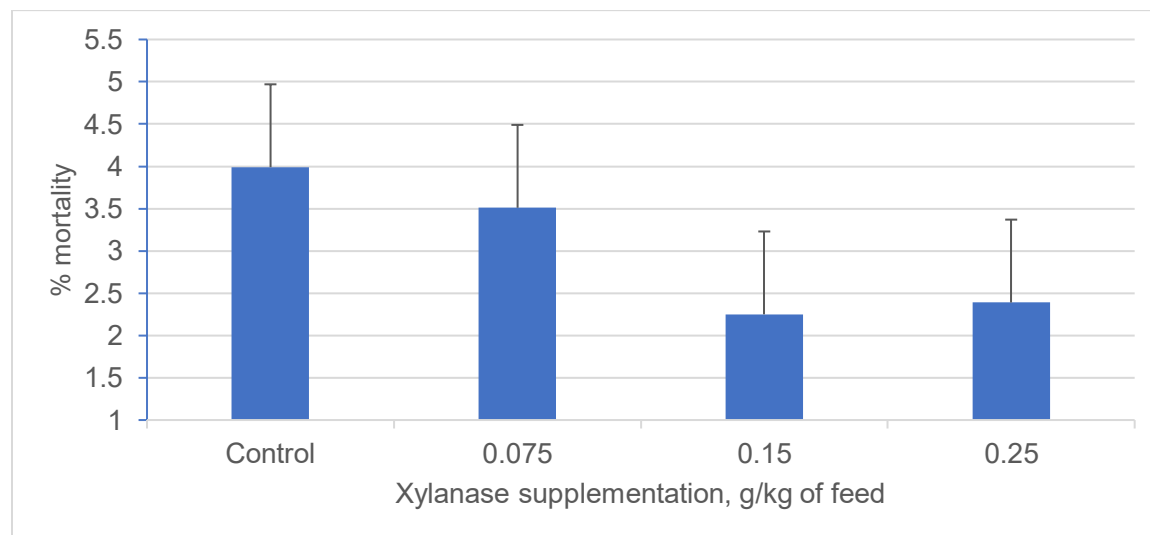
Figure 1. Effects of supplemental xylanase on mortality in grow-finishing pigs (Zier-Rush et al., 2016).

III. Low crude protein synthetic amino acids supplemented diets

In monogastric nutrition, protein (amino acids) is the second most expensive component of the feed after energy. The protein supply may have a significant impact on the intestinal microbiota, both qualitatively and quantitatively. High protein diets increase the concentrations of proteolytic

bacteria, especially *clostridia* and *E. coli* (Heo et al., 2013). From the viewpoint of animal health, it is interesting that there seems to be a link between enteric pathogens and certain protein sources. With respect to poultry, administration of feed with animal derived proteins led to a sharp increase in the concentrations of *Clostridium perfringens* and necrotic lesions in the intestinal mucosa (Drew et al., 2004). Adjusting protein supply and amino acid profiles can be considered as essential to achieve optimal performance and to control the intestinal formation of metabolites such as ammonia and biogenic amines from protein fermentation, that are generally considered detrimental (Nyachoti et al., 2006; Heo et al., 2013; Parenteau et al., 2020). The use of supplemental amino acids would offset or minimize the need to use some of expensive animal proteins, which could reduce the cost of feeds. Furthermore, extensive use of supplemental amino acids would allow to more precisely meet the animal dietary requirements while reducing dietary crude protein. This change in formulation can positively impact gut health and the environment by reduction of environmental excretion of nitrogen and reduce metabolic stress of detoxifying N-catabolites.

IV. Nurturing favorable microflora

Intensive rearing conditions do not usually allow for the natural microbial succession required for the establishment of a positive microbiota and the sufficient development of gut mucosal immune system (Friedman et al., 2012; Stanley et al., 2013). To optimize performance of pigs and poultry raised with AGP free feeding programs, it is essential to manage the composition of intestinal microbial community to avoid the inherent intestinal health risks of intensive production systems. In a drug free production system, the emphasis shifts from fighting the unfavorable organisms with antibiotics to nurturing the favorable organisms i.e. working with nature to ensure a favorable and stable intestinal ecology (see figure 2). (Collet, 2012) opined that the three most important legs of an effective intestinal management program includes "seeding" the gut with favorable organisms, "feeding" the favorable organisms and "weeding" out the unfavorable organisms.

Seeding the gut with favorable organisms: The first week after hatch for chicks or weaning for piglets is the most critical period. These young animals are susceptible to environmental and health challenges due to undeveloped digestive, thermoregulatory and immune systems. For example, colonization of mucosal surfaces in newly hatched chickens is dependent on environmental exposure mainly through feeds, litter, water etc (Stanley et al., 2013). Thus colonization of the gut with pioneer bacteria species, that are able to modulate expression of genes in the gut epithelia to optimize nutrient assimilation and create favorable conditions for establishment of a stable and beneficial climax flora, should be the starting point of any gut health management program (Collet, 2012). In this context, probiotics or direct fed microbials appear to be most effective during the initial development of the microbiota, or after any dietary change or stress and following antibiotic therapy and thus can be interpreted in the context of the ecological phenomena of primary and secondary succession in which a community is established or re-established following a disturbance (Collet, 2012). As methodological advancements continue, a progress toward development of novel probiotic approaches is plausible particularly in the area of probiotics with immunomodulation capabilities (Waititu et al., 2014; Neijat et al., 2019).
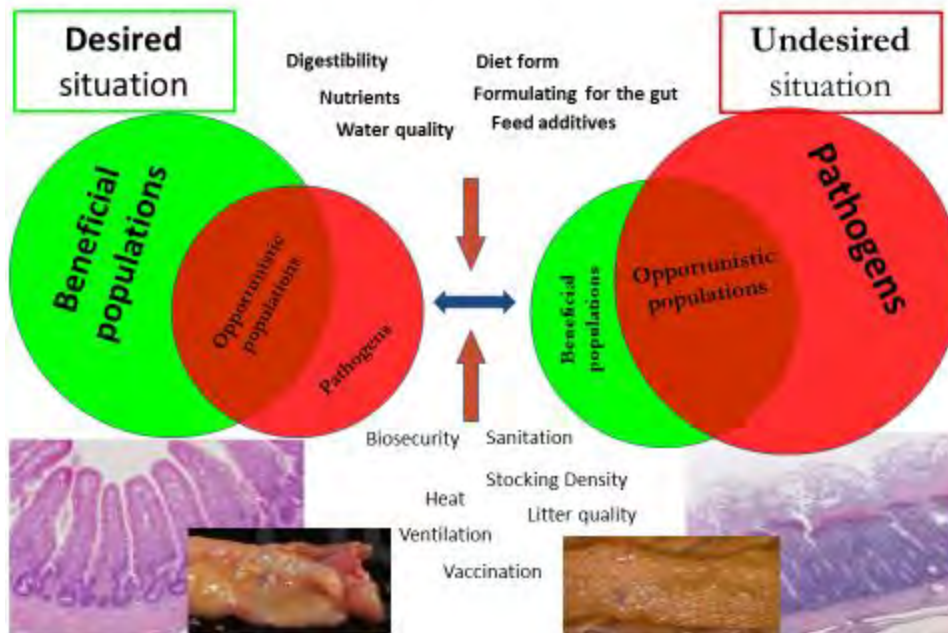
Figure 2. Strategies for maintaing a functional gut in the context of AGP feeding programs

Feeding the favorable organisms: In addition to seeding the gut with the correct pioneer species, it is crucial to enhance their ability to proliferate, compete and colonize to stall pathogen proliferation. There are many feed additives that could be used to promote proliferation of beneficial microbiome (Patterson and Burkholder, 2003). A major advantage of the symbiotic relationship between the microbiota and the host, is the ability of these organisms to provide energy to the intestinal epithelium in the form of SCFA from fibrous sources which is otherwise not digestible to the animal. Some oligosaccharides, such as inulin and oligofructose, yeast metabolites have been proposed as 'prebiotics' because of their potential to selectively stimulate growth of *Bifidobacterium* spp. within the human large intestine, suppress proliferation of potential pathogens and modulate a variety of human enteric conditions and diseases (Gibson and Roberfroid, 1995). Prebiotics are defined as 'non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and (or) activity of one or a limited number of bacteria in the colon, and hence improve host health' (Gibson and Roberfroid, 1995). The peculiarity with prebiotics is that they promote production of short chain fatty acids which are known to have a microbiota stabilizing effect and butyrate in particular has been shown to stimulate the production of host defense peptides (β-Defensins and Cathelicidins) (Sunkara et al., 2011). By providing a competitive advantage to the acid tolerant organisms such as the *Lactobacilli* and a competitive disadvantage to the acid intolerant organisms like the *Clostridia, Salmonella* and *E. coli* there is tremendous opportunity to maintain a functional and healthy gut. Such manipulation of the microbiota has both short and long term (Collet, 2012). It is important to keep in mind that a single layer of cells provide a barrier between the host and external pathogens and to maintain this barrier, the epithelial layer must be regenerated. The interaction between the

epithelial layer and microorganisms present in the GIT impacts the cell replacement rate and thus growth efficiency (Willing and Van Kessel, 2007).

Weeding out the unfavorable organisms: Nurmi and Rantala introduced the term competitive exclusion (CE) more than 4 decades ago following observation that oral gavage of newly hatched chicks with intestinal contents from *salmonella*-free adult birds reduced *Salmonella* colonization (Nurmi and Rantala, 1973). Competitive exclusion generally refers to a reduction in colonization by a pathogen due to several possible mechanisms: physical occupation of a site, resource competition in a physical or chemical niche, or direct physical or chemical insult to the potential colonist (Oakley et al., 2014). Although the underlying mechanisms remain poorly understood, pioneering work of Nurmi and Rantala (1973) has inspired development of several commercial products (Oakley et al., 2014). However, practical application remains elusive because undefined cultures are often more effective in controlling *salmonella* than the defined cultures in commercially regulated products (Oakley et al., 2014). Alternative strategies have capitalized on increasing understanding of the molecular basis that pathogens use to attach to the mucosal for colonization. Microbe attachment to host cell docking sites on the intestinal epithelium is dependent on surface molecule structure and this is the pivotal first step in the colonization and infection (Giron et al., 2002). For example, blocking the attachment mechanism of unfavorable organisms with a type-1 fimbria blocker can reduce their capacity to compete with the favorable organisms in the gut (Giron et al., 2002). Products that mimic docking sites for specific gut epithelia glycoproteins may be useful in preventing attachment and colonization by gut pathogens recognizing these sites (Giron et al., 2002). For example, several bacteria exhibit a binding effect specific for the sugar mannose (Mirelman et al., 1980). Mannose in the cell wall may cause the yeast or its residue to act as a decoy for the attachment of bacteria to the intestinal wall and this has been the basis of commercial success of many yeast based products (Kiarie et al., 2010; Kiarie et al., 2011; Kiarie et al., 2012; Corrigan et al., 2015).

References

Agyekum, A. K., B. A. Slominski, and C. M. Nyachoti. 2012. Organ weight, intestinal morphology, and fasting whole-body oxygen consumption in growing pigs fed diets containing distillers dried grains with solubles alone or in combination with a multienzyme supplement. J Anim Sci 90(9):3032-3040. doi: 10.2527/jas.2011-4380

Antoniou, T. C., and R. R. Marquardt. 1982. Utilization of Rye Diets by Chicks as Affected by Lipid Type and Level and Penicillin Supplementation. Poultry Sci 61(1):107-116.

Apajalahti, J., A. Kettunen, and H. Graham. 2004. Characteristics of the gastrointestinal microbial communities, with special reference to the chicken. World Poultry Sci J 60(2):223-232. doi: 10.1079/Wps200415

Apajalahti, J., A. Kettunen, and H. Graham. 2007. Characteristics of the gastrointestinal microbial communities, with special reference to the chicken. World's Poultry Science Journal 60(2):223-232. doi: 10.1079/WPS200415

Aviagen. 2019. Ross 708 broiler: Performance objectives. p 16. Aviagen, Huntsville, AL.

Bedford, M. R. 2018. The evolution and application of enzymes in the animal feed industry: the role of data interpretation. Brit Poultry Sci 59(5):486-493. doi: 10.1080/00071668.2018.1484074

Bedford, M. R., and A. J. Cowieson. 2012. Exogenous enzymes and their effects on intestinal microbiology. Animal Feed Science and Technology 173(1-2):76-85.

Cant, J. P., B. W. McBride, and W. J. Croom. 1996. The regulation of intestinal metabolism and its impact on whole animal energetics. Journal of Animal Science 74(10):2541-2553.

Collet, S. 2012. The "Seed, Feed and Weed" approach to managing intestinal health in drug free production systems The poultry informed professional No. 124, University of Georgia.

Corrigan, A., M. de Leeuw, S. Penaud-Frezet, D. Dimova, and R. A. Murphy. 2015. Phylogenetic and functional alterations in bacterial community compositions in broiler ceca as a result of mannan oligosaccharide supplementation. Appl Environ Microbiol 81(10):3460-3470. doi: 10.1128/AEM.04194-14

Courtin, C. M., W. F. Broekaert, K. Swennen, O. Lescroart, O. Onagbesan, J. Buyse, E. Decuypere, T. Van de Wiele, M. Marzorati, W. Verstraete, G. Huyghebaert, and J. A. Delcour. 2008. Dietary inclusion of wheat bran arabinoxylooligosaccharides induces beneficial nutritional effects in chickens. Cereal Chem 85(5):607-613. doi: 10.1094/Cchem-85-5-0607

Drew, M. D., N. A. Syed, B. G. Goldade, B. Laarveld, and A. G. Van Kessel. 2004. Effects of dietary protein source and level on intestinal populations of Clostridium perfringens in broiler chickens. Poult Sci 83(3):414-420.

Ferket, P. R. 2012. Embryo epigenomic response to breeder management and nutrition XXIV World's Poultry Congress. p 1-11, Salvador, Bahia, Brazil.

Friedman, A., O. Elad, I. Cohen, and E. Bar Shira. 2012. The Gut Associated Lymphoid System in the Post-Hatch Chick: Dynamics of Maternal IgA. Isr J Vet Med 67(2):75-81.

Gibson, G. R., and M. B. Roberfroid. 1995. Dietary Modulation of the Human Colonic Microbiota - Introducing the Concept of Prebiotics. J Nutr 125(6):1401-1412.

Giron, J. A., A. G. Torres, E. Freer, and J. B. Kaper. 2002. The flagella of enteropathogenic Escherichia coli mediate adherence to epithelial cells. Mol Microbiol 44(2):361-379.

Gous, R. M. 2010. Nutritional limitations on growth and development in poultry. Livest Sci 130(1-3):25-32. doi: 10.1016/j.livsci.2010.02.007

Havenstein, G. B., P. R. Ferket, and M. A. Qureshi. 2003. Growth, livability, and feed conversion of 1957 versus 2001 broilers when fed representative 1957 and 2001 broiler diets. Poultry Sci 82(10):1500-1508.

Heo, J. M., F. O. Opapeju, J. R. Pluske, J. C. Kim, D. J. Hampson, and C. M. Nyachoti. 2013. Gastrointestinal health and function in weaned pigs: a review of feeding strategies to control post-weaning diarrhoea without using in-feed antimicrobial compounds. J Anim Physiol Anim Nutr (Berl) 97(2):207-237. doi: 10.1111/j.1439-0396.2012.01284.x

Kaldhusdal, M., and E. Skjerve. 1996. Association between cereal contents in the diet and incidence of necrotic enteritis in broiler chickens in Norway. Preventive Veterinary Medicine 28(1):1-16. doi: https://doi.org/10.1016/0167-5877(96)01021-5

Kiarie, E., S. Bhandari, D. O. Krause, M. Scott, and C. M. Nyachoti. 2010. Weaned piglet responses to Escherichia coli K88+ oral challenge when fed diets containing a S. cerevisiae fermentation product with or without in-feed antibiotics. Journal of animal science 88 (E-Suppl. 2):653(Abstr.).

Kiarie, E., S. Bhandari, M. Scott, D. O. Krause, and C. M. Nyachoti. 2011. Growth performance and gastrointestinal microbial ecology responses of piglets receiving Saccharomyces cerevisiae fermentation products after an oral challenge with Escherichia coli (K88). J Anim Sci 89(4):1062-1078. doi: 10.2527/jas.2010-3424.

Kiarie, E., L. F. Romero, and C. M. Nyachoti. 2013. The role of added feed enzymes in promoting gut health in swine and poultry. Nutr Res Rev 26(1):71-88. doi: 10.1017/S0954422413000048.

Kiarie, E., M. Scott, D. O. Krause, H. Khazanehei, E. Khafipour, and C. M. Nyachoti. 2012. Interactions of Saccharomyces cerevisiae fermentation product and in-feed antibiotic on gastrointestinal and immunological responses in piglets challenged with Escherichia coli K88+. J Anim Sci 90 Suppl 4:1-3. doi: 10.2527/jas.52977.

Kiarie, E., C. Voth, D. Wey, C. Zhu, P. Vingerhoeds, S. Borucki, and E. J. Squires. 2018. Comparative efficacy of antibiotic growth promoter and benzoic acid on growth performance, nutrient utilization, and indices of gut health in nursery pigs fed corn–soybean meal diet. Can J Anim Sci 98(4):868-874. doi: 10.1139/cjas-2018-0056.

Kiarie, E., F. Waikibia, G. Kamande, and C. M. Nyachoti. 2019a. Utility of Saccharomyces cerevisiae fermentation product in pig feeding programs: amelioration of deleterious effects of enteric bacterial pathogens. In: Animal Production Soceity of Kenya (APSK) 2019 Scientific Symposium, Hotel Waterbuck, Nakuru, Kenya. p 41-45.

Kiarie, E., M. C. Walsh, and C. M. Nyachoti. 2016. Performance, digestive function, and mucosal responses to selected feed additives for pigs. J Anim Sci 94(supplement3):169-180. doi: 10.2527/jas.2015-9835.

Kiarie, E., M. C. Walsh, L. F. Romero, X. Yang, and S. K. Baidoo. 2015. Comparative efficacy of a blend of multiple enzymes and an in-feed antibiotic on growth performance and apparent digestibility of energy and protein in nursery pigs. Animal Production Science 55:1540.

Kiarie, E. G., H. Leung, R. Akbari Moghaddam Kakhki, R. Patterson, and J. R. Barta. 2019b. Utility of Feed Enzymes and Yeast Derivatives in Ameliorating Deleterious Effects of Coccidiosis on Intestinal Health and Function in Broiler Chickens. Frontiers in Veterinary Science 6(473)(Review) doi: 10.3389/fvets.2019.00473.

Kiarie, E. G., and A. Mills. 2019. Role of Feed Processing on Gut Health and Function in Pigs and Poultry: Conundrum of Optimal Particle Size and Hydrothermal Regimens. Frontiers in Veterinary Science 6(19)(Review) doi: 10.3389/fvets.2019.00019.

Kim, E., H. Leung, N. Akhtar, J. Li, J. R. Barta, Y. Wang, C. Yang, and E. Kiarie. 2017. Growth performance and gastrointestinal responses of broiler chickens fed corn-soybean meal diet without or with exogenous epidermal growth factor upon challenge with Eimeria. Poultry Sci 96(10):3676-3686.

Krupa, E., Z. Krupová, M. Wolfová, and E. Žáková. 2017. Estimation of economic values for traits of pig breeds in different breeding systems: II. Model application to a three-way crossing system. Livest Sci 205:70-78. doi: 10.1016/j.livsci.2017.09.018.

Leung, H., A. Yitbarek, R. Snyder, R. Patterson, J. R. Barta, N. Karrow, and E. Kiarie. 2019. Responses of broiler chickens to Eimeria challenge when fed a nucleotide-rich yeast extract1. Poultry Sci:pey533-pey533. doi: 10.3382/ps/pey533.

Lindemann, M. D., S. G. Cornelius, S. M. el Kandelgy, R. L. Moser, and J. E. Pettigrew. 1986. Effect of age, weaning and diet on digestive enzyme levels in the piglet. J Anim Sci 62(5):1298-1307.

Marquardt, R. R., A. T. Ward, and R. Misir. 1979. The Retention of Nutrients by Chicks Fed Rye Diets Supplemented with Amino Acids and Penicillin. Poultry Sci 58(3):631-640. doi: https://doi.org/10.3382/ps.0580631.

Mateos, G. G., E. Jimenez-Moreno, M. P. Serrano, and R. P. Lazaro. 2012. Poultry response to high levels of dietary fiber sources varying in physical and chemical characteristics. J Appl Poultry Res 21(1):156-174. doi: 10.3382/japr.2011-00477.

Merks, J. W. M. 2018. One century of genetic changes in pigs and the future needs. BSAP Occasional Publication 27:8-19. doi: 10.1017/S1463981500040498.

Mirelman, D., G. Altmann, and Y. Eshdat. 1980. Screening of bacterial isolates for mannose-specific lectin activity by agglutination of yeasts. J Clin Microbiol 11(4):328-331. .

Munyaka, P. M., N. K. Nandha, E. Kiarie, C. M. Nyachoti, and E. Khafipour. 2016. Impact of combined beta-glucanase and xylanase enzymes on growth performance, nutrients utilization and gut microbiota in broiler chickens fed corn or wheat-based diets. Poult Sci 95(3):528-540. doi: 10.3382/ps/pev333.

Neijat, M., J. Habtewold, R. B. Shirley, A. Welsher, J. Barton, P. Thiery, and E. Kiarie. 2019. Bacillus subtilis Strain DSM 29784 Modulates the Cecal Microbiome, Concentration of Short-Chain Fatty Acids, and Apparent Retention of Dietary Components in Shaver White Chickens during Grower, Developer, and Laying Phases. Appl Environ Microb 85(14):e00402-00419. doi: 10.1128/aem.00402-19.

NRC. 1994. Nutrient Requirements of Poultry. 9th revision ed. National Academy of Sciences Press, Washington, D.C.

NRC. 2012. Nutrient Requirements of Swine. 11th revision ed. National Academy of Sciences Press, Washington, D.C.

Nurmi, E., and M. Rantala. 1973. New aspects of Salmonella infection in broiler production. Nature 241(5386):210-211.

Nyachoti, C. M., F. O. Omogbenigun, M. Rademacher, and G. Blank. 2006. Performance responses and indicators of gastrointestinal health in early-weaned pigs fed low-protein amino acid-supplemented diets. J Anim Sci 84(1):125-134.

Oakley, B. B., H. S. Lillehoj, M. H. Kogut, W. K. Kim, J. J. Maurer, A. Pedroso, M. D. Lee, S. R. Collett, T. J. Johnson, and N. A. Cox. 2014. The chicken gastrointestinal microbiome. FEMS Microbiol Lett 360(2):100-112. doi: 10.1111/1574-6968.12608.

Parenteau, I. A., M. Stevenson, and E. G. Kiarie. 2020. Egg production and quality responses to increasing isoleucine supplementation in Shaver white hens fed a low crude protein corn-soybean meal diet fortified with synthetic amino acids between 20 and 46 weeks of age. Poultry Sci 99(3):1444-1453. doi: https://doi.org/10.1016/j.psj.2019.10.064.

Patterson, J. A., and K. M. Burkholder. 2003. Application of prebiotics and probiotics in poultry production. Poultry Sci 82(4):627-631.

Pluske, J. R. 2016. Invited review: Aspects of gastrointestinal tract growth and maturation in the pre- and postweaning period of pigs. Journal of Animal Science 94:399-411. doi: 10.2527/jas2015-9767.

Pluske, J. R., D. W. Pethick, D. E. Hopwood, and D. J. Hampson. 2002. Nutritional influences on some major enteric bacterial diseases of pig. Nutr Res Rev 15(2):333-371. doi: 10.1079/NRR200242.

Ravindran, V. 2012. Advances and Future Directions in Poultry Nutrition: An Overview. Korean J Poult Sci 39:53-62. .

Slominski, B. A. 2011. Recent advances in research on enzymes for poultry diets. Poult Sci 90(9):2013-2023. doi: 10.3382/ps.2011-01372.

Smulders, A. C. J. M., Veldman A. and Enting H. 1999. Effect of anti-microbial growth promoter in feeds with different levels of indigestible protein on broiler performance. In: 12th European Symposium on Poultry Nutrition, Veldhaven, The Netherlands.

Stanley, D., M. S. Geier, R. J. Hughes, S. E. Denman, and R. J. Moore. 2013. Highly Variable Microbiota Development in the Chicken Gastrointestinal Tract. Plos One 8(12)doi: ARTN e84290.

Sunkara, L. T., M. Achanta, N. B. Schreiber, Y. R. Bommineni, G. Dai, W. Jiang, S. Lamont, H. S. Lillehoj, A. Beker, R. G. Teeter, and G. Zhang. 2011. Butyrate enhances disease resistance of chickens by inducing antimicrobial host defense peptide gene expression. PLoS One 6(11):e27225. doi: 10.1371/journal.pone.0027225.

Timbermont, L., F. Haesebrouck, R. Ducatelle, and F. Van Immerseel. 2011. Necrotic enteritis in broilers: an updated review on the pathogenesis. Avian Pathology 40(4):341-347. doi: 10.1080/03079457.2011.590967.

Uni, Z., and R. P. Ferket. 2004. Methods for early nutrition and their potential. World Poultry Sci J 60(1):101-111. doi: 10.1079/Wps20038.

Urriola, P. E., and H. H. Stein. 2012. Comparative digestibility of energy and nutrients in fibrous feed ingredients fed to Meishan and Yorkshire pigs. J Anim Sci 90(3):802-812. doi: 10.2527/jas.2010-3254.

Waititu, S. M., A. Yitbarek, E. Matini, H. Echeverry, E. Kiarie, J. C. Rodriguez-Lecompte, and C. M. Nyachoti. 2014. Effect of supplementing direct-fed microbials on broiler performance, nutrient digestibilities, and immune responses. Poultry Sci 93(3):625-635. doi: 10.3382/ps.2013-03575.

Wealleans, A. L., M. C. Walsh, L. F. Romero, and V. Ravindran. 2017. Comparative effects of two multi-enzyme combinations and a Bacillus probiotic on growth performance, digestibility of energy and nutrients, disappearance of non-starch polysaccharides, and gut microflora in broiler chickens. Poultry Sci 96(12):4287-4297. doi: 10.3382/ps/pex226.

Xu, Y., C. R. Stark, P. R. Ferket, C. M. Williams, W. J. Pacheco, and J. Brake. 2015. Effect of dietary coarsely ground corn on broiler live performance, gastrointestinal tract development, apparent ileal digestibility of energy and nitrogen, and digesta particle size distribution and retention time. Poultry Sci 94(1):53-60. doi: 10.3382/ps/peu015.

Zier-Rush, C. E., C. Groom, M. Tillman, J. Remus, and R. D. Boyd. 2016. The feed enzyme xylanase improves finish pig viability and carcass feed efficiency. Journal of Animal Science 94:115-115. doi: 10.2527/msasas2016-244

Diet complexity and L-threonine supplementation: effects on growth performance, immune response, and intestinal integrity in nursery pigs

Complexité du régime alimentaire et supplémentation en L-thréonine : effets sur la performance de croissance, la réponse immunitaire et l'intégrité intestinale chez les porcs en pouponnière

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Abstract

Despite higher economic value of a simplified nursery pig diet compared to a conventional complex diet, a simple diet may stimulate the pig's immune system and impair intestinal integrity. Dietary Thr supplementation is required when pigs are immunologically challenged to maintain the gut health. Thus, a study was performed to investigate the effects of diet complexity and L-Thr supplementation on growth performance, immune response and gut integrity in nursery pigs. Thirty-two pigs (body weight 7.23 ± 0.48 kg) were randomly assigned to dietary treatments in a 2×2 factorial arrangement based on diet complexity (complex or simple) and dietary Thr content. The complex diet contained fish meal, plasma protein and dried whey to mimic a conventional nursery diet. The simple diet was formulated with corn, wheat, and soybean meal. L-Thr was supplemented to each diet to supply either 100% (STD Thr) or 115% (SUP Thr) of the NRC (2012) requirement for Thr. Pigs were individually housed and fed the diets ad libitum for 14 d. All data were analyzed using the MIXED procedure of SAS. Diet complexity, dietary Thr content, and their interactions were considered main effects. Dietary treatment did not affect growth performance. However, pigs fed the simple diet had greater ($P < 0.05$) plasma interleukin (IL)-10 and IL-6 concentrations compared to those fed the complex diet on d 7 and d 14, respectively. Simple diet-fed pigs tended to show greater ($P < 0.10$) expression of genes encoding for tumor necrosis factor-α, claudin-1, and zonula occludens-1 in the jejunum compared to complex diet-fed pigs. The SUP Thr increased ($P < 0.05$) villus height and goblet cell density in villi and crypts in the jejunum and deepened ($P < 0.05$) crypts in the proximal colon. The SUP Thr resulted in the upregulation ($P < 0.05$) of occludin gene expression and tended to downregulate ($P = 0.10$) IL-6 gene expression in the jejunum. Interactions ($P < 0.05$) between diet complexity and L-Thr supplementation level were observed in goblet cell density in the crypt in the jejunum. In conclusion, SUP Thr in a simple diet would be a nutritional strategy to fortify the gut integrity in nursery pig production, but it does not prevent the induction of inflammation caused by simple diet in nursery pigs.

Résumé

Malgré la valeur économique plus élevée d'un régime alimentaire simplifié pour les porcs en pouponnière comparativement à un régime complexe conventionnel, le premier peut stimuler le système immunitaire de l'animal et nuire à son intégrité intestinale. Pour préserver la santé intestinale des porcs présentant une déficience immunologique, la supplémentation en Thr est nécessaire. Une étude a donc été réalisée pour vérifier les effets de la complexité du régime alimentaire et de la supplémentation en L-Thr sur la performance de croissance, la réponse immunitaire et l'intégrité intestinale des porcs en pouponnière. Trente-deux porcs (poids corporel de 7,23 ± 0,48 kg) ont été assignés de manière aléatoire à des traitements diététiques selon un arrangement factoriel 2 × 2 basé sur la complexité du régime alimentaire (complexe ou simple) et la teneur en L-Thr. Le régime complexe contenait de la farine de poisson, des protéines plasmatiques et du lactosérum déshydraté, imitant ainsi un régime conventionnel de pouponnière. Le régime simple a été formulé avec du maïs, du blé et du tourteau de soya. De la L-Thr a été ajoutée à chaque régime pour fournir soit 100 % (STD Thr) soit 115 % (SUP Thr) des besoins en Thr recommandés par le NRC (2012). Les porcs ont été logés individuellement et nourris ad libitum pendant 14 jours. Toutes les données ont été analysées en utilisant la procédure MIXTE du SAS. La complexité du régime alimentaire, la teneur en Thr et leurs interactions ont été considérées comme les principaux effets. Le traitement alimentaire n'a pas influencé la performance de croissance. Cependant, les porcs nourris avec le régime simple ont présenté des concentrations plasmatiques d'interleukine (IL)-10 et IL-6, respectivement aux jours 7 et 14, plus élevées ($P < 0,05$) que ceux nourris avec le régime complexe. Les porcs nourris avec le régime simple ont eu tendance à présenter une plus grande ($P < 0,10$) expression des gènes codant pour le facteur de nécrose tumorale-α, la claudine-1 et les *zonula occludens* dans le jéjunum que les porcs nourris avec le régime complexe. Le traitement SUP Thr a augmenté ($P < 0,05$) la hauteur des villosités et la densité des cellules à gobelet des villosités et des cryptes du jéjunum et a creusé ($P < 0,05$) les cryptes du côlon proximal. Le traitement SUP Thr a entraîné une régulation à la hausse ($P < 0,05$) de l'expression génétique de l'occludine et a eu tendance à réguler à la baisse ($P = 0,10$) l'expression génétique de l'IL-6 dans le jéjunum. Des interactions ($P < 0,05$) entre la complexité du régime alimentaire et le niveau de supplémentation en L-Thr ont été observées dans la densité des cellules à gobelet dans les cryptes du jéjunum. En conclusion, la supplémentation en thréonine d'un régime alimentaire simple serait une stratégie nutritionnelle permettant de renforcer l'intégrité intestinale chez les porcs en pouponnière, mais elle n'empêche pas le déclenchement de l'inflammation causée par un régime simple chez les porcs en pouponnière.

Introduction

Nursery pig diets have been conventionally formulated with animal protein sources and dairy products (e.g., fish meal, plasma meal, and whey protein). However, this has resulted in a complex diet composition and high feed costs. Many attempts have therefore been made to simplify the conventional complex diet by increasing the proportion of soybean meal as a way to save on feed costs in nursery pig production. Previous studies (Skinner et al. 2014; Koo et al. 2017) confirmed that simple diets do not compromise the growth performance at the end of the nursery phase.

However, concerns remain that the large amounts of dietary fiber, fermentable protein, and antigenic compounds in soybean meal may stimulate the pig's immune system and impair intestinal integrity (Koo et al. 2017). This may result in poor growth performance in pigs raised in a commercial swine barn environment (Pastorelli et al. 2012). Threonine is a major component of mucins and γ-globulins (Wang et al. 2009). Previous studies have reported that dietary L-Thr supplementation over the NRC (2012) recommended levels improved intestinal morphology and immune status by regulating immunoglobulins and cytokines in pigs. We hypothesized that pigs fed a simple diet would elicit systemic and gut inflammation and show impairment in their gut integrity and barrier function when compared with pigs fed a complex diet and that L-Thr supplementation would ameliorate the inflammation and the gut impairment. The objective of this study was to investigate the effects of diet complexity and L-Thr supplementation levels on the growth performance, immune response, intestinal integrity, and microbial metabolites in nursery pigs.

Methods

Thirty-two male piglets (TN 70 × TN Tempo; Topigs Norsvin, Winnipeg, MB) with an initial body weight of 7.23 ± 0.48 kg (28-d-old) were randomly assigned to a 2×2 factorial arrangement based on diet complexity and the levels of dietary L-Thr supplementation. Pigs were individually housed for 14 d and had *ad libitum* access to feed and water. The complex diet contained animal protein sources (e.g., fish meal, spray-dried animal plasma) and a dairy product (e.g., dried whey) to mimic a conventional nursery diet. These animal protein sources and dairy product were replaced with soybean meal (30.3%) to make the simple diet. The two respective diets were supplemented with L-Thr to supply the standard NRC (2012) level of Thr (STD Thr) or 15% over the standardized ileal digestible (SID) Thr requirement (SUP Thr) for pigs weighing 9 kg. On day 7 and 14, blood samples from the jugular vein of all the pigs were collected for determination of inflammatory cytokine concentrations. On day 14, all pigs were euthanized to collect jejunum and colon samples and their contents for morphology, mRNA gene expression, and volatile fatty acids (VFA) and ammonia-nitrogen (NH_3-N). All data were analyzed using the MIXED procedure of SAS (version 9.4; SAS Inst. Inc., Cary, NC, USA) with each animal used as the experimental unit. The model included diet complexity, the level of L-Thr supplementation, and their interaction.

Results

Pigs fed the simple diet had greater ($P < 0.05$) plasma interleukin (IL)-10 and IL-6 concentrations compared to those fed the complex diet on day 7 and day 14, respectively (Table 1). Simple diet-fed pigs tended to show greater ($P < 0.10$) expression of genes encoding for tumor necrosis factor-α, claudin-1, and zonula occludens-1 in the jejunum compared to complex diet-fed pigs (Figure 1). The simple diet–fed pigs had greater ($P < 0.05$) concentrations of NH_3-N in the jejunum digesta than did complex diet–fed pigs (Table 1).

Table 1. Effect of diet complexity and Thr supplementation on plasma cytokine concentrations, jejunal morphology, and microbial metabolites in jejunum and colon digesta.

Item[1]	Complex		Simple		SEM	P-value		
	STD Thr	SUP Thr	STD Thr	SUP Thr		Diet	Thr	Diet × Thr
Plasma IL-6, pg/mL	555	945	2,297	2,211	688.6	0.04	0.83	0.73
Plasma IL-10, pg/mL	112	45	239	245	74.7	0.03	0.66	0.60
Jejunum								
VH, μm	345	375	347	393	17.9	0.55	0.02	0.59
VH:CD	1.5^{xy}	1.6^{xy}	1.4^{y}	1.8^{x}	0.09	0.52	0.03	0.07
GC/100 μm villi	2.5^{y}	2.8^{xy}	2.2^{y}	3.8^{x}	0.35	0.31	<0.05	0.06
GC/100 μm crypt	6.7^{y}	6.8^{xy}	6.1^{y}	8.0^{x}	0.35	0.32	<0.01	0.01
Total VFA in colon	239.0^{xy}	188.4^{y}	224.3^{xy}	249.2^{x}	14.69	0.106	0.359	0.011
Ammonia-N in jejunum	73.6^{y}	93.8^{xy}	158.5^{x}	76.3^{y}	18.82	0.07	0.09	0.01

[1]IL, interleukin; VH, villus height; CD, crypt depth; GC, goblet cells; VFA, volatile fatty acids.
x,yWithin a row, means with different superscripts differ ($P < 0.05$).
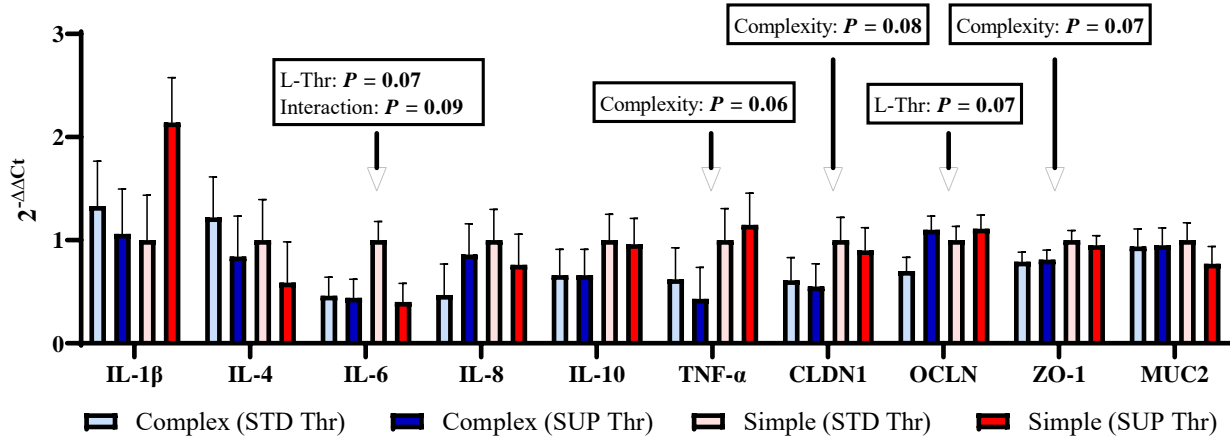
Figure 1. Effect of diet complexity and Thr supplementation on relative mRNA gene expression ($2^{-\Delta\Delta Ct}$) in jejunum. The values were expressed. The gene expression values are expressed in relation to the simple-STD Thr diet group. IL = interleukin; TNF-α = tumor necrosis factor alpha; CLDN1 = claudin 1; OCLN = occludin; ZO-1 = zonula 1; MUC2 = mucin 2.

SUP Thr increased ($P < 0.05$) the density of goblet cells, where mucin is secreted, in villi as well as in crypts in the jejunum, regardless of diet complexity. Furthermore, SUP Thr–group showed higher villi and upregulated ($P = 0.04$) occludin (a tight junction protein) in the jejunum, regardless of diet complexity. Evidence is growing that dietary Thr supplementation beneficially modifies the gut microbiota composition because the stimulated mucins (glycoconjugates) can serve as substrates for microorganisms (Chen et al. 2017). Interestingly, SUP Thr in the simple diet decreased ($P < 0.05$) NH_3 content in the jejunum to the level observed for the complex diet. Conversely, SUP Thr in the simple diet increased ($P < 0.05$) total VFA production in the colon compared to that in the complex diet. The SUP Thr tended to suppress ($P = 0.07$) the expression of IL-6 in the jejunum, but the suppressing effect was larger with a simple diet (Interaction $P = 0.10$). However, there was no difference in plasma IL-6 and IL-10 concentrations between the SUP Thr and the STD Thr groups. Despite various changes in gut health indicators, no differences in the growth performance of pigs were observed among dietary treatments.

Conclusion

Feeding a simple diet to nursery pigs resulted in systemic and intestinal inflammation. SUP Thr decreased NH_3 production in the jejunum and resulted in downregulation of IL-6 gene expression in the jejunum. SUP Thr also improved gut integrity and architecture. The benefits of SUP Thr seems to be greater with a simple diet than with a complex diet. Therefore, SUP Thr in combination with a simple diet could be a beneficial cost-saving strategy while maintaining gut health.

References

Chen, Y.P., Cheng, Y.F., Li, X.H., Yang, W.L., Wen, C., Zhuang, S., and Zhou, Y.M. 2017. Effects of threonine supplementation on the growth performance, immunity, oxidative status, intestinal integrity, and barrier function of broilers at the early age. Poult. Sci. **96**: 405-413. doi: 10.3382/ps/pew240

Koo, B., Kim, J.W., de Lange, C.F.M., Hossain, M.M., and Nyachoti, C.M. 2017. Effects of diet complexity and multicarbohydrase supplementation on growth performance, nutrient digestibility, blood profile, intestinal morphology, and fecal score in newly weaned pigs. J. Anim. Sci. **95**: 4060-4071. doi: 10.2527/jas2017.1760

NRC. 2012. Nutrient Requirements of Swine 11th rev. ed. Natl. Acad. Press, Washigton, DC, USA.

Pastorelli, H., van Milgen, J., Lovatto, P., and Montagne, L. 2012. Meta-analysis of feed intake and growth responses of growing pigs after a sanitary challenge. Animal **6**: 952-961. doi: 10.1017/S175173111100228X

Skinner, L.D., Levesque, C.L., Wey, D., Rudar, M., Zhu, J., Hooda, S., and de Lange, C.F.M. 2014. Impact of nursery feeding program on subsequent growth performance, carcass quality, meat quality, and physical and chemical body composition of growing-finishing pigs. J. Anim. Sci. **92**: 1044-1054. doi: 10.2527/jas.2013-6743

Wang, W.W., Qiao, S.Y., and Li, D.F. 2009. Amino acids and gut function. Amino Acids **37**: 105-110. doi: 10.1007/s00726-008-0152-4

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Non-antibiotic Strategies to Reduce Inflammation in Poultry

Stratégies non antibiotiques pour réduire l'inflammation chez les volailles

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Abstract

Poultry hatch with an under-developed acquired immune system, and rely heavily on innate immunity. However, inflammation can divert nutrients and energy away from growth and production. Strategies to reduce the intensity and duration of inflammation, and transition towards the maturing acquired immune system can allow for efficient production while maintaining bird health. Antibiotic growth promotors (AGP) directly or indirectly reduced systemic inflammation caused by microbes within the digestive tract, thus maintaining efficient production. As the poultry industry moves away from the use of AGP, successful alternative strategies will also manage systemic inflammation. Although individual products may show promise in laboratory or controlled production settings, translation to the field has been less successful. A potential reason is that AGP provided a broad protection against performance-reducing organisms. Although individual replacements may be effective against a particular group of problem organisms, or under specific conditions, no single product has yet been an effective one-for-one replacement of AGP. Therefore, a combination of different product types, with different mechanisms, may be required to match the broad-based effectiveness of AGP. Additionally, the optimum combination of alternatives may vary from company to company, location to location, and season to season over time. The transition away from AGP has been successful in many places, including Canada, but has generally involved a methodical evaluation of various combinations of alternative products. By understanding the mechanisms of each alternative approach, and the specific challenges faced on each farm, a strategic approach can be used to effectively transition from AGP use.

Résumé

Les volailles naissent avec un système immunitaire acquis sous-développé et dépendent fortement de l'immunité innée. Cependant, l'inflammation peut détourner les nutriments et l'énergie au détriment de la croissance et de la production. Les stratégies visant à réduire l'intensité et la durée de l'inflammation et la transition vers le système immunitaire acquis en cours de maturation peuvent permettre une production efficace tout en préservant la santé des oiseaux. Les antibiotiques stimulateurs de croissance réduisent directement ou indirectement l'inflammation systémique causée par les microbes dans le tube digestif, maintenant ainsi une production efficace. À mesure que l'industrie avicole abandonne l'utilisation des antibiotiques stimulateurs de croissance, d'autres stratégies efficaces permettront également de combattre l'inflammation systémique. Bien que certains produits individuels semblent prometteurs en laboratoire ou en milieu contrôlé, la transposition sur le terrain a été moins réussie. Une raison possible est que les antibiotiques stimulateurs de croissance fournissent une protection contre un large spectre d'organismes nuisibles pour la performance. Bien qu'individuellement, des substituts puissent être efficaces contre un groupe particulier d'organismes dommageables, ou dans des conditions spécifiques, aucun produit n'a encore remplacé efficacement un antibiotique stimulateur de croissance. Par conséquent, une combinaison de différents types de produits comportant des mécanismes différents pourrait être nécessaire pour égaler l'efficacité des antibiotiques stimulateurs de croissance en général. En outre, la combinaison optimale de solutions de remplacement peut varier d'une entreprise à l'autre, d'un endroit à l'autre et d'une saison à l'autre au fil du temps. L'abandon progressif des antibiotiques stimulateurs de croissance a été couronné de succès dans de nombreux endroits, y compris au Canada, mais a généralement reposé sur une évaluation méthodique de diverses combinaisons de produits de substitution. En comprenant les mécanismes de chaque méthode de remplacement ainsi que les défis spécifiques rencontrés dans chaque exploitation agricole, une approche stratégique peut être utilisée pour réussir à produire efficacement sans le recours aux antibiotiques stimulateurs de croissance.

Introduction

The immune system of poultry is typically divided into innate responses and adaptive responses, and reviews of the avian immune system can be found elsewhere (Korver, 2012; Kogut, et al., 2020). Briefly, the innate response includes non-specific mechanisms of exclusion such as the skin and mucosal surfaces of the digestive tract, lungs and other organ systems with exposure to the outside world. Thus, one of the primary means of immunological protection is the prevention the interaction of potential pathogens with the internal environment of the animal. Should a foreign organism gain contact with the internal environment through a break in the skin or disruption of the intestinal barrier, non-specific innate immune mechanisms at the site of infection (e.g. local inflammation, recruitment of phagocytic cells, etc.) and potentially, systemic physiological changes (e. g. systemic inflammation, fever, anorexia, cachexia, etc.) are activated. Thus, the innate immune response is an essential, but potentially costly means of host protection.

Unlike the innate response, the adaptive immune response works against specific target antigens (Kogut, et al., 2020). In order for a strong adaptive response to occur, a primary exposure to a pathogen must be recognized (in a general sense, as being "foreign") by the innate immune system, and antigens presented to the cells of the adaptive response. Future exposure to the same antigen

can then result in a rapid, specific response to counteract continued infection. This is the strategy employed in vaccination programs. Broiler chicks hatch with a limited adaptive immune response (Bar-Shira, et al., 2003; Bar-Shira and Friedman, 2005; Bar-Shira and Friedman, 2006; Friedman, et al., 2007), and as such rely heavily on innate immunity for the first part of life (Alkie, et al., 2019).

The adaptive immune response works in conjunction with the innate response. Foreign antigens are processed and presented by cells of the innate immune system to cells of the adaptive immune system (Sylte and Suarez, 2012; Chhabra, et al., 2015). The vaccination response requires an initial activation of the innate immune system (Schijns, et al., 2014), which comes at an energetic and nutritional cost associated with the initial involvement of innate immunity. Therefore, vaccination against specific diseases must be viewed in the context of the cost of the innate response on performance vs the likelihood of a particular disease being encountered by the flock.

General Strategies to Replace Antibiotic Growth Promotors

Antibiotics have used to increase chicken growth rates since 1940's (Moore, et al., 1946; Castanon, 2007).   Although a number of mechanisms have been proposed (Visek, 1978; Thomke and Elwinger, 1998; Dibner and Richards, 2005), it appears that ultimately, the growth promoting (i.e. allowing the birds to express a greater proportion of their genetic potential) effects are primarily mediated through a reduction in intestinal (Oh, et al., 2019) and systemic inflammation (Roura, et al., 1992; Niewold, 2007). Therefore, it is reasonable to conclude that successful strategies to replace AGP will also involve the direct or indirect reduction of inflammation through reducing the local and systemic effects of inflammation.

The removal of antibiotic growth promotors (AGP) increases the risk of subclinical and clinical bacterial infections that can result in the diversion of nutrients away from growth in support of the systemic inflammatory response (Broom and Kogut, 2018). The physiologic changes associated with systemic inflammation such as reduced feed intake, anorexia, cachexia, fever, etc. reduce the amount of nutrients available for growth (Iseri and Klasing, 2013a; Iseri and Klasing, 2013b; Iseri and Klasing, 2014).

The changes in metabolism and physiology associated with a continued activation of a systemic innate response will decrease the productivity of broilers. Therefore, a holistic approach to managing bird health while reducing or eliminating antibiotic growth promotors in broiler production should include:

- **Reduce exposure to challenges that will initiate systemic inflammation**. Bacterial infections can reduce the growth and efficiency of broiler chickens (Remus, et al., 2014). In the context of AGP replacement strategies, this means reducing the immunological interaction between the host and potential pathogens. This can be done by reducing the prevalence of potential pathogens, maintaining a healthy barrier function of the gut, and using products that prevent proliferation and invasion of tissues by pathogenic bacteria. Even low-grade intestinal inflammation can lead to reduced performance (Dal Pont, et al.,

2020). Ultimately, one of the main effects of AGP is to reduce these interactions, and decrease systemic inflammation.

- **Rapid resolution of systemic inflammation.** The innate response is essential for the bird's well-being, but excessive activation, or extended activation will reduce performance. The more quickly the innate response can be resolved, the lower the impact on performance. Alternatively, reducing the systemic effects of inflammation while maintaining a strong local response can achieve the same end goal (Korver and Klasing, 1997; Korver, et al., 1997).
- **Rapid transition from innate to adaptive immunity.** The innate response is essential for the initial recognition of a pathogen, but a transition from innate to an effective adaptive response will decrease the overall impact of immune activation (Broom and Kogut, 2018).

Evaluating Replacements for Antibiotic Growth Promotors

When evaluating the potential of an individual product or multi-product strategy to replace AGP, it is essential that the supporting research be conducted appropriately. The simplest, but incorrect approach to evaluating a potential AGP replacement is to run an experiment in which two diets are fed: a positive control containing an AGP, and a test diet containing the test product, but no AGP. A lack of difference in performance is interpreted as evidence that the product can successfully replace AGP. However, in low-challenge research environments, the inclusion or exclusion of an AGP may have no effect on broiler performance, since in the absence of a bacterial challenge, there is no opportunity for the AGP to act. In very clean environments, there will be little inflammation, even in the absence of AGP (Roura, et al., 1992). Therefore, it cannot be concluded whether the replacement would have an effect in the case of a challenge. In field research, however, it may be impractical to include a true negative control, since commercial producers are not likely to accept the potential of substantial economic losses inherent in a true negative control treatment. In such cases, it may only be possible to include a positive control, and a test product for which there is already a strong indication of effectiveness from smaller-scale studies. To increase the confidence in the outcomes of field studies like this, all treatments should be included within a statistical block (i.e. a production unit -- within individual barns, or across barns at a single location), and the number of blocks should be replicated as often as is feasible, either across locations, or across time.

In addition to sound experimental design principles, the following criteria are essential for controlled AGP-replacement research studies:

- **A positive control treatment containing a relevant AGP**. Depending on the product(s) being tested, and the target disease challenge being study, the PC may or may not have a coccidiostat included.
- **A negative control treatment without AGP, and without any test product**. In order to be able to draw valid conclusions about efficacy, there must be a reduction in performance in this group relative to the PC. This provides evidence that there is a challenge in the experimental environment, and that the inclusion of an AGP mitigates this challenge. In the absence of reduced performance in the negative control group, it is impossible to evaluate the effects of a test product, because in this situation the AGP had no effect on

performance. Often this approach includes the use of a clinical or sub-clinical challenge model to all treatment groups, including the positive control and test groups.

- **One or more experimental treatments based on the negative control diet, but containing the product(s) of interest**. As noted above, it is necessary for the positive control group to have superior performance to the negative control group. If performance of the test group is statistically identical to that of the negative control, it can be concluded that the product has no effect. If the test group has performance greater than the negative control group, but statistically similar to the positive control, it can be concluded that, under the conditions of the experiment, the test product was effective as an AGP. If performance of the test group is intermediate to the positive and negative controls, it can be concluded that, under the experimental conditions, the product could be a partial replacement for AGP, but additional strategies may be needed to obtain equivalent protection as provided by AGP.

Although results may show promise in small-scale, well-controlled experiments, it is necessary to proceed with caution before declaring a product or combination to be an effective replacement for AGP. The results obtained in highly-controlled research facilities may translate with limited success to commercial conditions.

Specific Approaches to Replacing Antibiotic Growth Promotors

Although many potential AGP replacements have been proposed, experience has shown that success is not as simple as a one-for-one replacement of AGP with another product.

Additionally, with a narrower specificity than AGP, it is important to identify the specific types of challenges that are likely at particular times of life, and strategically apply products intended to address those particular issues. So, rather than removing AGP from each dietary phase and replacing a single product or combination of products throughout the life of the bird, it may be necessary to transition from one product or group of products to another over time as the type of challenge changes.

A large number of products or compounds have been proposed as alternatives to antibiotics. It is not the author's intention to review all proposed alternatives, but rather to give a broad overview of products (Table 1). The efficacy of individual approaches or combinations of approaches need to be evaluated in the context of the published literature, as well as experience in the field. Immunomodulatory nutrients may also play a part in the overall strategy of a move away from growth promoting antibiotics, since they can influence the systemic responses to inflammatory challenges (Korver, 1997; Korver and Klasing, 1997; Korver, et al., 1997; Wils-Plotz, et al., 2013; Wils-Plotz and Klasing, 2015; Wils-Plotz and Klasing, 2017), but are reviewed elsewhere (Klasing, 2007; Adedokun and Olojede, 2019; Swaggerty, et al., 2019).

Table 1. Examples of products or strategies showing promise as AGP replacements.

Product or strategies	Putative mode of action	References
Probiotics	Competitive exclusion; control of inflammation and effects on immune system; production of antimicrobial peptides/bioactive molecules; microbiome influence on immune development.	(Yang, et al., 2009; Gaggìa, et al., 2010; Indira, et al., 2019; Ramlucken, et al., 2020; Rodrigues, et al., 2020; Tarradas, et al., 2020)
Prebiotics, including mannan oligosaccharides (MOS), fructo-ologisaccharides (FOS) xylo-oligosaccharides (XOS), chitin oligosachharides (COS), inulin.	Selectively stimulate growth of specific classes of bacteria; Prevention of pathogen adherence to intestinal cells; fermentation to short-chain fatty acids.	(Spring, 2000; Baurhoo, et al., 2012; Ricke, 2015; Gadde, et al., 2017; Lee, et al., 2017; Li, et al., 2019; Osho and Adeola, 2019; Pascual, et al., 2020)
Yeast products, including live yeast, MOS, β-glucans, chitin, nucleotides	Immune modulating effects; prebiotic; intestinal health; reduced pathogenic bacteria colonization.	(Xue, et al., 2017; Araujo, et al., 2018; Leung, et al., 2019; Massacci, et al., 2019; Elliott, et al., 2020; Pascual, et al., 2020)
Phytogenics, including essential oils, polyphenols and other plant extracts	Reduce counts of intestinal pathogens/antibacterial effects; increased secretion of digestive enzymes and nutrient digestibility; antioxidant activity; anti-inflammatory; disrupt quorum sensing.	(Yang, et al., 2015; Hafeez, et al., 2016; Gadde, et al., 2017; Upadhaya and Kim, 2017; Mehdi, et al., 2018; Reis, et al., 2018; Stevanovic, et al., 2018; Galli, et al., 2020; Granstad, et al., 2020)
Enzymes, including Non-starch polysaccharidases, phytase, β-mannanase, muramidase	Reduce nutrient availability to pathogens, create less favourable intestinal environment for pathogens, destruction of immunogenic molecules.	(Jackson, et al., 2004; Zhang, et al., 2010; Attia, et al., 2011; Arsenault, et al., 2017; Broom, 2017; Kiarie, et al., 2019)
Organic acids	Altered microbial community; lowered intestinal pH; action against cell wall of Gram -ve bacteria; stimulating growth of beneficial bacteria.	(Gadde, et al., 2017; Polycarpo, et al., 2017; Dittoe, et al., 2018; Mehdi, et al., 2018; Granstad, et al., 2020; Hu, et al., 2020; Pham, et al., 2020)
Butyrate	Control of enteric pathogens; reduced inflammation; increased gut barrier function	(Ahsan, et al., 2016; Bedford and Gong, 2018; Wu, et al., 2018)
Egg yolk antibodies	Bind to pathogens in the gastrointestinal tract to prevent colonization	(Chalghoumi, et al., 2009; Yegani and Korver, 2010; Gadde, et al., 2017)
Medium chain fatty acids	Induce host defense peptide expression; antimicrobial activity.	(Batovska, et al., 2009; Hejdysz, et al., 2018; Zhou, et al., 2019)
Bacteriophage	Direct antimicrobial activity	(Gadde, et al., 2017; Heo, et al., 2018; Gigante and Atterbury, 2019)
Antimicrobial peptides	Direct antimicrobial effects	(Gadde, et al., 2017; Daneshmand, et al., 2019; Wang, et al., 2020)

The path forward to consistent success in AGP-free broiler chicken production will likely depend on a combination of products with differing mechanisms of action. Making this more difficult is the likelihood that different geographical regions, or different growing facilities within a region may need slightly different combinations or approaches. Although individual products may show promise in laboratory or controlled production settings, translation to the field has been less successful. A potential reason is that AGP provided a broad protection against performance-reducing organisms. Although individual replacements may be effective against a particular group of problem organisms, or under specific conditions, no single product has yet been an effective on-for-one replacement of AGP. Therefore, a combination of different product types, with different mechanisms, may be required to match the broad-based effectiveness of AGP.

It is important to note that excellent animal care and husbandry will become even more important. AGP replacements will likely be less forgiving than AGP towards poor management or sub-standard environments. Novel strategies will be part of an overall program to maintain optimum health, since even AGP cannot overcome every possible disease challenge.

The broiler industry worldwide is transitioning, or has already transitioned to AGP-free production, with varying degrees of success. The evaluation of novel AGP replacement products must follow sound scientific principles, with robust experimental design and data analysis. Transition to commercial use should follow only after a demonstration of effectiveness under field conditions. Individual producers should be aware of the specific issues associated with the age of the bird, and their geographical location, and choose strategies to address those issues. Development of a farm-specific approach will likely take trial and error, and will likely take several years to develop. However, continual progress can be made with identification of candidate products, robust testing under controlled conditions, strategic testing under field conditions, and continual evaluation and improvements as new products and combinations become available.

References

Adedokun, S. A., and O. C. Olojede. 2019. Optimizing gastrointestinal integrity in poultry: the role of nutrients and feed additives. Front. Vet. Sci. 5:11.

Ahsan, U., O. Cengiz, I. Raza, E. Kuter, M. F. A. Chacher, Z. Iqbal, S. Umar, and S. Cakir. 2016. Sodium butyrate in chicken nutrition: the dynamics of performance, gut microbiota, gut morphology, and immunity. World's Poult. Sci. J. 72:265-275.

Alkie, T. N., A. Yitbarek, D. C. Hodgins, R. R. Kulkarni, K. Taha-Abdelaziz, and S. Sharif. 2019. Development of innate immunity in chicken embryos and newly hatched chicks: a disease control perspective. Avian Pathol. 48:288-310.

Araujo, L. F., M. Bonato, R. Barbalho, C. S. S. Araujo, P. S. Zorzetto, C. A. Granghelli, R. J. G. Pereira, and A. J. T. Kawaoku. 2018. Evaluating hydrolyzed yeast in the diet of broiler breeder hens. J. Appl. Poult. Res 27:65-70.

Arsenault, R. J., J. T. Lee, R. Latham, B. Carter, and M. H. Kogut. 2017. Changes in immune and metabolic gut response in broilers fed beta-mannanase in beta-mannan-containing diets. Poult. Sci. 96:4307-4316.

Attia, Y. A., H. S. Zeweil, A. A. Alsaffar, and A. S. El-Shafy. 2011. Effect of non-antibiotic feed additives as an alternative to flavomycin on productivity, meat quality and blood parameters in broilers. Archiv Geflugelk. 75:40-48.

Bar-Shira, E., and A. Friedman. 2005. Ontogeny of gut associated immune competence in the chick. Israel J. Vet. Med. 60:42-50.

Bar-Shira, E., and A. Friedman. 2006. Development and adaptations of innate immunity in the gastrointestinal tract of the newly hatched chick. Dev. and comparative immunology 30:930-941.

Bar-Shira, E., D. Sklan, and A. Friedman. 2003. Establishment of immune competence in the avian GALT during the immediate post-hatch period. Developmental Comp. Immunol. 27:147-157.

Batovska, D. I., T. Todorova, V. Tsvetkova, and H. M. Najdenski. 2009. Antibacterial study of the medium chain fatty acids and their 1-monoglycerides: individual effects and synergistic relationships. Polish J. Microbiol. 58:43-47.

Baurhoo, B., P. Ferket, C. M. Ashwell, J. de Oliviera, and X. Zhao. 2012. Cell walls of *Saccharomyces cerevisiae* differentially modulated innate immunity and glucose metabolism during late systemic inflammation. PloS one 7.

Bedford, A., and J. Gong. 2018. Implications of butyrate and its derivatives for gut health and animal production. Anim. Nutr. 4:151-159.

Broom, L. J. 2017. Necrotic enteritis; current knowledge and diet-related mitigation. World's Poult. Sci. J. 73:281-291.

Broom, L. J., and M. H. Kogut. 2018. Inflammation: friend or foe for animal production? Poult. Sci. 97:510-514.

Castanon, J. 2007. History of the use of antibiotic as growth promoters in European Poult. feeds. Poult. Sci. 86:2466-2471.

Chalghoumi, R., Y. Beckers, D. Portetelle, and A. Thewis. 2009. Hen egg yolk antibodies (IgY), production and use for passive immunization against bacterial enteric infections in chicken: a review. Biotechnol. Agron. Soc. 13:295-308.

Chhabra, R., J. Chantrey, and K. Ganapathy. 2015. Immune responses to virulent and vaccine strains of infectious bronchitis viruses in chickens. Viral Immunol. 28:478-488.

Dal Pont, G. C., M. Farnell, Y. Farnell, and M. H. Kogut. 2020. Dietary factors as triggers of low-grade chronic intestinal inflammation in poultry. Microorganisms 8:10.

Daneshmand, A., H. Kermanshahi, M. H. Sekhavati, A. Javadmanesh, and M. Ahmadian. 2019. Antimicrobial peptide, cLF36, affects performance and intestinal morphology, microflora, junctional proteins, and immune cells in broilers challenged with *E. coli.* Scientific Rep. 9:9.

Dibner, J. J., and J. D. Richards. 2005. Antibiotic growth promoters in agriculture: history and mode of action. Poult. Sci. 84:634-643.

Dittoe, D. K., S. C. Ricke, and A. S. Kiess. 2018. Organic acids and potential for modifying the avian gastrointestinal tract and reducing pathogens and disease. Front. Vet. Sci. 5:12.

Elliott, K. E. C., S. L. Branton, J. D. Evans, S. A. Leigh, E. J. Kim, H. A. Olanrewaju, G. T. Pharr, H. O. Pavlidis, P. D. Gerard, and E. D. Peebles. 2020. Growth and humoral immune effects of dietary Original XPC in layer pullets challenged with *Mycoplasma gallisepticum*. Poult. Sci. *in press*. doi https://doi.org/10.1016/j.psj.2020.01.016

Friedman, A., E. Bar-shira, and D. Sklan. 2007. Ontogeny of gut associated immune competence in the chick. World's Poult. Sci. J. 59:209-219.

Gadde, U., W. H. Kim, S. T. Oh, and H. S. Lillehoj. 2017. Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry: a review. Anim. Health Res. Rev. 18:26-45.

Gaggìa, F., P. Mattarelli, and B. Biavati. 2010. Probiotics and prebiotics in animal feeding for safe food production. Int. J. Food Microbiol. 141:S15-S28.

Galli, G. M., R. R. Gerbet, L. G. Griss, B. F. Fortuoso, T. G. Petrolli, M. M. Boiago, C. F. Souza, M. D. Baldissera, J. Mesadri, R. Wagner, G. da Rosa, R. E. Mendes, A. Gris, and A. S. Da Silva. 2020. Combination of herbal components (curcumin, carvacrol, thymol, cinnamaldehyde) in broiler chicken feed: Impacts on response parameters, performance, fatty acid profiles, meat quality and control of coccidia and bacteria. Microb. Pathog. 139:11.

Gigante, A., and R. J. Atterbury. 2019. Veterinary use of bacteriophage therapy in intensively-reared livestock. Virol. J. 16:9.

Granstad, S., A. B. Kristoffersen, S. L. Benestad, S. K. Sjurseth, B. David, L. Sorensen, A. Fjermedal, D. H. Edvardsen, G. Sanson, A. Lovland, and M. Kaldhusdal. 2020. Effect of feed additives as alternatives to in-feed antimicrobials on production performance and intestinal *Clostridium perfringens* counts in broiler chickens. Animals 10:19.

Hafeez, A., K. Männer, C. Schieder, and J. Zentek. 2016. Effect of supplementation of phytogenic feed additives (powdered vs. encapsulated) on performance and nutrient digestibility in broiler chickens. Poult. Sci. 95:622-629.

Hejdysz, M., S. Kaczmarek, D. Jozefiak, D. Jamroz, and A. Rutkowski. 2018. Effect of different medium chain fatty acids, calcium butyrate, and salinomycin on performance, nutrient utilization, and fermentation products in gastrointestinal tracts of broiler chickens. J. Anim. Plant Sci. 28:377-387.

Heo, S., M. G. Kim, M. Kwon, H. S. Lee, and G. B. Kim. 2018. Inhibition of *Clostridium perfringens* using bacteriophages and bacteriocin producing strains. Korean J. Food Sci. Anim. Resour. 38:88-98.

Hu, Y., L. D. Wang, D. Shao, Q. Wang, Y. Y. Wu, Y. M. Han, and S. R. Shi. 2020. Selected and reshaped early dominant microbial community in the cecum with similar proportions and better homogenization and species diversity due to organic acids as AGP alternatives mediate their effects on broilers growth. Front. Microbiol. 10:20.

Indira, M., T. C. Venkateswarulu, K. A. Peele, M. N. Bobby, and S. Krupanidhi. 2019. Bioactive molecules of probiotic bacteria and their mechanism of action: a review. 3 Biotech 9:11.

Iseri, V. J., and K. C. Klasing. 2013a. The cost of an immune response to *Escherichia coli* in *Gallus gallus*. Integr. Comp. Biol. 53:E100-E100.

Iseri, V. J., and K. C. Klasing. 2013b. Dynamics of the systemic components of the chicken (*Gallus gallus domesticus*) immune system following activation by *Escherichia coli*; implications for the costs of immunity. Dev. Comp. Immunol. 40:248-257.

Iseri, V. J., and K. C. Klasing. 2014. Changes in the amount of lysine in protective proteins and immune cells after a systemic response to dead *Escherichia coli*: Implications for the nutritional costs of immunity. Integr. Comp. Biol. 54:922-930.

Jackson, M., K. Geronian, A. Knox, J. McNab, and E. McCartney. 2004. A dose-response study with the feed enzyme b-mannanase in broilers provided with corn-soybean meal based diets in the absence of antibiotic growth promoters. Poult. Sci. 83:1992-1996.

Kiarie, E. G., H. Leung, R. A. M. Kakhki, R. Patterson, and J. R. Barta. 2019. Utility of feed enzymes and yeast derivatives in ameliorating deleterious effects of coccidiosis on intestinal health and function in broiler chickens. front. vet. sci. 6:13.

Klasing, K. C. 2007. Nutrition and the immune system. Br. Poult. Sci. 48:525-537.

Kogut, M. H., A. Lee, and E. Santin. 2020. Microbiome and pathogen interaction with the immune system. Poult. Sci. 99:1906-1913.

Korver, D. 1997. Modulation of the growth suppressing effects of inflammation by the use of dietary fatty acids. Ph. D. Dissertation. University of California, Davis.

Korver, D. R. 2012. Implications of changing immune function through nutrition in poultry. Anim. Feed Sci. Technol. 173:54-64.

Korver, D. R., and K. C. Klasing. 1997. Dietary fish oil alters specific and inflammatory immune responses in chicks. J. Nutr. 127:2039-2046.

Korver, D. R., P. Wakenell, and K. C. Klasing. 1997. Dietary fish oil or lofrin, a 5-lipoxygenase inhibitor, decrease the growth-suppressing effects of coccidiosis in broiler chicks. Poult. Sci. 76:1355-1363.

Lee, S. A., J. Apajalahti, K. Vienola, G. Gonzalez-Ortiz, C. Fontes, and M. R. Bedford. 2017. Age and dietary xylanase supplementation affects ileal sugar residues and short chain fatty acid concentration in the ileum and caecum of broiler chickens. Anim. Feed Sci. Technol. 234:29-42.

Leung, H., A. Yitbarek, R. Snyder, R. Patterson, J. R. Barta, N. Karrow, and E. Kiarie. 2019. Responses of broiler chickens to *Eimeria* challenge when fed a nucleotide-rich yeast extract. Poult. Sci. 98:1622-1633.

Li, J., Y. F. Cheng, Y. P. Chen, H. M. Qu, Y. R. Zhao, C. Wen, and Y. M. Zhou. 2019. Dietary chitooligosaccharide inclusion as an alternative to antibiotics improves intestinal morphology, barrier function, antioxidant capacity, and immunity of broilers at early age. Animals 9:12.

Massacci, F. R., C. Lovito, S. Tofani, M. Tentellini, D. A. Genovese, A. A. P. De Leo, P. Papa, C. F. Magistrali, E. Manuali, M. Trabalza-Marinucci, L. Moscati, and C. Forte. 2019. Dietary *Saccharomyces cerevisiae boulardii* CNCM I-1079 positively affects performance and intestinal ecosystem in broilers during a *Campylobacter jejuni* infection. Microorganisms 7:21.

Mehdi, Y., M. P. Letourneau-Montminy, M. L. Gaucher, Y. Chorfi, G. Suresh, T. Rouissi, S. K. Brar, C. Cote, A. A. Ramirez, and S. Godbout. 2018. Use of antibiotics in broiler production: Global impacts and alternatives. Anim. Nutr. 4:170-178.

Moore, P. R., A. Evenson, T. D. Luckey, E. McCoy, E. A. Elvehjem, and E. B. Hart. 1946. Use of sulphasuccidine, streptothricin and streptomycin in nutrition studies with the chick. J. Biol. Chem. 165:437–441.

Niewold, T. A. 2007. The nonantibiotic anti-inflammatory effect of antimicrobial growth promoters, the real mode of action? A hypothesis. Poult. Sci. 86:605-609.

Oh, S., H. S. Lillehoj, Y. Lee, D. Bravo, and E. P. Lillehoj. 2019. Dietary antibiotic growth promoters down-regulate intestinal inflammatory cytokine expression in chickens challenged with LPS or co-infected with *Eimeria maxima* and *Clostridium perfringens*. Front. Vet. Sci. 6:13.

Osho, S. O., and O. Adeola. 2019. Impact of dietary chitosan oligosaccharide and its effects on coccidia challenge in broiler chickens. Br. Poult. Sci. 60:766-776.

Pascual, A., M. Pauletto, M. Giantin, G. Radaelli, C. Ballarin, M. Birolo, C. Zomeno, M. Dacasto, M. Bortoletti, M. Vascellari, G. Xiccato, and A. Trocino. 2020. Effect of dietary supplementation with yeast cell wall extracts on performance and gut response in broiler chickens. J. Anim. Sci. Biotechnol. 11:11.

Pham, V. H., L. G. Kan, J. Y. Huang, Y. Q. Geng, W. R. Zhen, Y. M. Guo, W. Abbas, and Z. Wang. 2020. Dietary encapsulated essential oils and organic acids mixture improves gut health in broiler chickens challenged with necrotic enteritis. J. Anim. Sci. Biotechnol. 11:18.

Polycarpo, G. V., I. Andretta, M. Kipper, V. C. Cruz-Polycarpo, J. C. Dadalt, P. H. M. Rodrigues, and R. Albuquerque. 2017. Meta-analytic study of organic acids as an alternative performance-enhancing feed additive to antibiotics for broiler chickens. Poult. Sci. 96:3645-3653.

Ramlucken, U., S. O. Ramchuran, G. Moonsamy, R. Lalloo, M. S. Thantsha, and C. J. van Rensburg. 2020. A novel *Bacillus* based multi-strain probiotic improves growth performance and intestinal properties of *Clostridium perfringens* challenged broilers. Poult. Sci. 99:331-341.

Reis, J. H., R. R. Gebert, M. Barreta, M. D. Baldissera, I. D. dos Santos, R. Wagner, G. Campigotto, A. M. Jaguezeski, A. Gris, J. L. F. de Lima, R. E. Mendes, M. Fracasso, M. M. Boiago, L. M. Stefani, D. S. dos Santos, W. S. Robazza, and A. S. Da Silva. 2018. Effects of phytogenic feed additive based on thymol, carvacrol and cinnamic aldehyde on body weight, blood parameters and environmental bacteria in broilers chickens. Microb. Pathog. 125:168-176.

Remus, A., L. Hauschild, I. Andretta, M. Kipper, C. R. Lehnen, and N. K. Sakomura. 2014. A meta-analysis of the feed intake and growth performance of broiler chickens challenged by bacteria. Poult. Sci. 93:1149-1158.

Ricke, S. C. 2015. Potential of fructooligosaccharide prebiotics in alternative and nonconventional poultry production systems. Poult Sci 94:1411-1418.

Rodrigues, D. R., K. M. Wilson, M. Trombetta, W. N. Briggs, A. F. Duff, K. M. Chasser, W. G. Bottje, and L. Bielke. 2020. A proteomic view of the cross-talk between early intestinal microbiota and poultry immune system. Front. Physiol. 11:13.

Roura, E., J. Homedes, and K. C. Klasing. 1992. Prevention of immunologic stress contributes to the growth-permitting ability of dietary antibiotics in chicks. J. Nutr. 122:2383-2390.

Schijns, V. E. J. C., S. van de Zande, B. Lupiani, and S. M. Reddy. 2014. Chapter 20 - Practical Aspects of Poultry Vaccination. Pages 345-362 in: Avian Immunology (Second Edition). K. A. Schat, B. Kaspers, and P. Kaiser eds. Academic Press, Boston.

Spring, P. C. Wenk, K. A. Dawson, and K. E. Newman. 2000. The effects of dietary mannanoligosaccharides on cecal parameters and the concentrations of enteric bacteria in the ceca of Salmonella-challenged broiler chicks. Poult. Sci. 79:205-211.

Stevanovic, Z. D., J. Bosnjak-Neumuller, I. Pajic-Lijakovic, J. Raj, and M. Vasiljevic. 2018. Essential oils as feed additives - future perspectives. Molecules 23.

Swaggerty, C. L., T. R. Callaway, M. H. Kogut, A. Piva, and E. Grilli. 2019. modulation of the immune response to improve health and reduce foodborne pathogens in poultry. Microorganisms 7:10.

Sylte, M. J., and D. L. Suarez. 2012. Vaccination and acute phase mediator production in chickens challenged with low pathogenic avian influenza virus; novel markers for vaccine efficacy? Vaccine 30:3097-3105.

Tarradas, J., N. Tous, E. Esteve-Garcia, and J. Brufau. 2020. The control of intestinal inflammation: a major objective in the research of probiotic strains as alternatives to antibiotic growth promoters in poultry. Microorganisms 8:16.

Thomke, S., and K. Elwinger. 1998. Growth promotants in feeding pigs and poultry. II. Mode of action of antibiotic growth promotants. Ann. Zootech. 47:153-167.

Upadhaya, S. D., and I. H. Kim. 2017. Efficacy of phytogenic feed additive on performance, production and health status of monogastric animals - a review. Ann. Anim. Sci. 17:929-948.

Visek, W. 1978. The mode of growth promotion by antibiotics. J. Anim. Sci. 46:1447-1469.

Wang, G., Q. L. Song, S. Huang, Y. M. Wang, S. Cai, H. T. Yu, X. L. Ding, X. F. Zeng, and J. Zhang. 2020. Effect of antimicrobial peptide microcin J25 on growth performance, immune regulation, and intestinal microbiota in broiler chickens challenged with *Escherichia coli* and *Salmonella*. Animals 10:11.

Wils-Plotz, E., and K. Klasing. 2015. Immune response to a *Salmonella enteritidis* infection in chickens fed different immunomodulatory nutrients. FASEB J. 29:1.

Wils-Plotz, E. L., M. C. Jenkins, and R. N. Dilger. 2013. Modulation of the intestinal environment, innate immune response, and barrier function by dietary threonine and purified fiber during a coccidiosis challenge in broiler chicks. Poult. Sci. 92:735-745.

Wils-Plotz, E. L., and K. C. Klasing. 2017. Effects of immunomodulatory nutrients on growth performance and immune-related gene expression in layer chicks challenged with lipopolysaccharide. Poult. Sci. 96:548-555.

Wu, W., Z. B. Xiao, W. Y. An, Y. Y. Dong, and B. K. Zhang. 2018. Dietary sodium butyrate improves intestinal development and function by modulating the microbial community in broilers. PloS one 13:21.

Xue, G. D., S. B. Wu, M. Choct, and R. A. Swick. 2017. Effects of yeast cell wall on growth performance, immune responses and intestinal short chain fatty acid concentrations of broilers in an experimental necrotic enteritis model. Anim. Nutr. 3:399-405.

Yang, C. B., M. A. K. Chowdhury, Y. Q. Hou, and J. Gong. 2015. Phytogenic compounds as alternatives to in-feed antibiotics: Potentials and challenges in application. Pathogens 4:137-156.

Yang, Y., P. Iji, and M. Choct. 2009. Dietary modulation of gut microflora in broiler chickens: a review of the role of six kinds of alternatives to in-feed antibiotics. World's Poult. Sci. J. 65:97-114.

Yegani, M., and D. R. Korver. 2010. Application of egg yolk antibodies as replacement for antibiotics in poultry. World's Poult. Sci. J. 66:27-37.

Zhang, G., G. F. Mathis, C. L. Hofacre, P. Yaghmaee, R. A. Holley, and T. D. Duranc. 2010. Effect of a radiant energy-treated lysozyme antimicrobial blend on the control of clostridial necrotic enteritis in broiler chickens. Avian Dis. 54:1298-1300.

Zhou, Z. X., J. Huang, H. H. Hao, H. K. Wei, Y. F. Zhou, and J. Peng. 2019. Applications of new functions for inducing host defense peptides and synergy sterilization of medium chain fatty acids in substituting in-feed antibiotics. J. Funct. Foods 52:348-359.

Nutrient Absorption and Health in Young Calves

Absorption des nutriments et santé des jeunes veaux

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Abstract

Newborn calves are born with a rumen that is underdeveloped and non-functioning. By the time calves are weaned, the rumen is the principal site for VFA absorption. From birth to weaning, the rumen must undergo a physiological transformation to develop its VFA absorptive capacity, important for growth, health, and productivity throughout a calf's life. Nutritional strategies to promote rumen development typically focus on feeding rapidly fermentable feeds that increase production of butyrate, a key driver of rumen papillae development. Recently, studies have shown more complexity in rumen development and VFA absorption capacity than was previously assumed. At weaning, overall VFA absorption capacity is similar regardless of papillae development, so the development and adaptation of the cells that make up the rumen epithelium play a key role in rumen development. Furthermore, weaning develops the rumen microbiome and epithelium, regardless of age. Post-weaning, the rumen microbiome and epithelium do not yet physiologically resemble those of a mature cow, so development of nutrient absorption capacity in calves extends well past weaning. More insight is needed into nutrition and management practices during weaning and beyond to promote nutrient absorption and health in calves. A better understanding of rumen development during early stages of transition will likely have immediate impacts on growth and further lifelong impacts on productivity.

Résumé

Les veaux naissent avec un rumen sous-développé et non fonctionnel. Au moment du sevrage des veaux, le rumen est le principal site d'absorption des AGV. De la naissance au sevrage, le rumen doit subir une transformation physiologique pour développer sa capacité d'absorption des AGV, un mécanisme important pour la croissance, la santé et la productivité tout au long de la vie du veau. Les stratégies nutritionnelles visant à promouvoir le développement du rumen

se concentrent généralement sur l'utilisation d'aliments rapidement fermentescibles qui augmentent la production de butyrate, un facteur clé du développement des papilles du rumen. Récemment, des études ont montré que le développement du rumen et l'accroissement de la capacité d'absorption des AGV étaient plus complexes qu'on ne le pensait auparavant. Au sevrage, la capacité d'absorption globale des AGV est similaire, quel que soit le développement des papilles; ainsi, le développement et l'adaptation des cellules qui composent l'épithélium ruminal jouent un rôle clé dans le développement du rumen. En outre, le sevrage favorise le développement du microbiome et de l'épithélium du rumen, quel que soit l'âge. Après le sevrage, il faut un certain temps au microbiome et à l'épithélium du rumen pour ressembler physiologiquement à ceux d'une vache adulte, de sorte que le développement de la capacité d'absorption des nutriments chez les veaux se poursuit bien au-delà du sevrage. Il est nécessaire de mieux comprendre les pratiques de nutrition et de régie au sevrage et après pour favoriser l'absorption des nutriments et la santé chez les veaux. Une meilleure connaissance du développement du rumen au cours des premières étapes de la transition aura probablement des effets immédiats sur la croissance et d'autres effets à plus long terme sur la productivité.

Introduction

At birth, the rumen is non-functional, making calves pre-ruminants. By weaning, the rumen is the principal site of volatile fatty acid (**VFA**) absorption, responsible for over 50% of all VFA being absorbed (Allen, 1997). Consequently, the calf rumen must undergo a ruminant transition, where the rumen goes from non-functional to functional in 6-10 weeks, coinciding with the period when most dairy calves are weaned (USDA, 2012). The transition of the rumen from non-functional to principal site of energy absorption is the biggest physiological change the rumen will undergo, therefore requiring considerable attention and management.

A functional rumen requires an intact and functional epithelium. To be fully functional, an epithelium must carry out two principal functions: 1) the exchange of nutrients across the epithelial barrier; and 2) preventing the translocation of bacteria into the bloodstream. During the ruminant transition, changes in nutrient absorption capacity and epithelial barrier integrity occur simultaneously, so nutrient absorption and health are closely linked in early life during the ruminant transition of rumen development.

Rumen Development

In a neonatal calf, the rumen wall is smooth, with little vasculature and musculature (**Figure 1**). The development of papillae and vasculature stems predominantly from the consumption and fermentation of rapidly fermentable calf starter, which calves will begin to eat in the second or third week of life. Through fermentation of calf starter, microbes in the rumen will produce butyrate, the VFA most effective at stimulating rumen development (Warner et al., 1956). As the link between calf starter intake and rumen development was discovered, maximizing calf starter intake became the primary goal of calf raising(USDA, 2012). In the NRC (2001) for dairy cattle, weaning of calves is recommended once daily calf starter intake is 680 g/day (1.5 lb/day), though this threshold was recently adjusted to 1000 g/day (Stamey et al., 2012). Crucially, calf starter intake is the only weaning indicator currently recommended.

Figure 1. Rumen development at 28 days of age of calves fed milk replacer only (LEFT) and milk replacer with calf starter (RIGHT). Calves fed calf starter show more rumen development, evidenced by dark, prominent papillae, which increase absorptive surface area of the rumen. Modified from https://extension.psu.edu/photos-of-rumen-development.

To maximize calf starter intake, feeding a low plane of nutrition (low amounts of milk or milk replacer) was popular. When calves were fed a low plane of nutrition of 750 g/d, their calf starter intake reached 680 g/d by 7 weeks of age (Laarman and Oba, 2011). In another study, calf starter intake increased once the weaning transition began, regardless of whether calves were fed a high or low plane of nutrition, or whether they were weaned at five or eight weeks of age (de Passillé et al., 2011). What these studies show is that starter intake and milk intake are inversely linked. Furthermore, milk and starter play two distinct roles and are digested and absorbed at different sites.

When suckled from a bottle, milk bypasses the rumen via the esophageal groove and is shunted directly to the omasum and abomasum. In the abomasum, milk is coagulated via rennin, and then further digested and absorbed in the small intestine. The absorption of nutrients from milk is key to meeting the calf's energy needs early in life. Feeding colostrum, for instance, increases lactase activity needed to digest and absorb lactose in milk (Hammon et al., 2012). Additionally, fat oxidation increases dramatically in the first day of life, especially medium chain fatty acids most common in milk fats (Girard et al., 1992). At birth, the calf is therefore geared.(Girard et al., 1992). Consequently, closure of the esophageal groove, abomasal rennin, and lactase activity in the small intestine are crucial for meeting the calf's energy needs until the shift to solid feed is complete.

Unlike milk, calf starter does not use the small intestine as the primary site for digestion and absorption. Instead, calf starter is primarily digested, fermented, and absorbed in the rumen, although rumen-undegradable-protein, -fat, and -fibre bypass the rumen and are still digested and absorbed in the lower gut. The difference in primary site of digestion and absorption, however, does have big implications for the importance of optimal rumen function in a weaned calf. For the rumen to become the principal site of nutrient absorption, it must develop the rumen epithelium from birth to weaning.

If the rumen is insufficiently developed prior to weaning, nutrient absorption is compromised and growth suffers. In one study, scientists weaned calves completely at 42 days of age, with the weaning transition lasting anywhere from 0-22 days (Sweeney et al., 2010). In calves where weaning transition began at day 20 of life, the decrease in milk intake led to an increase in calf starter intake, as expected (**Figure 2**). Even though calf starter intake in the 22-day weaning group ate as much starter as the 10-day weaning group, the total digestible energy intake and body weight never kept up.
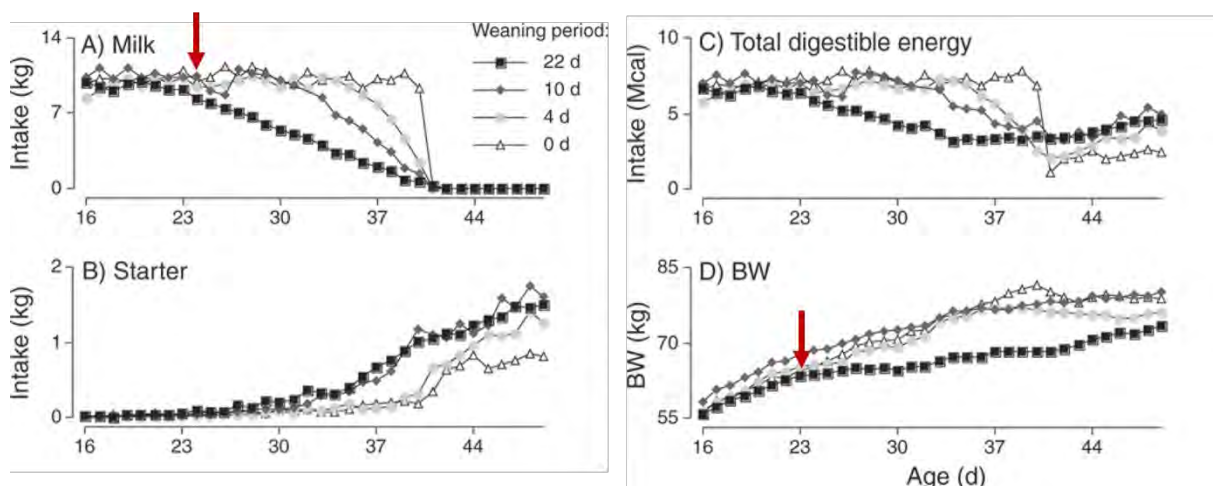
Figure 2. Impact of premature weaning on growth. Calves whose weaning transition began on day 21 (A; black squares) increased their starter intake (B) but were unable to make up for the loss of milk, resulting in lower energy intake (C) and lower growth (D). Modified from Sweeney et al. (2010).

Over time, the rumen's ability to ingest, digest, and absorb nutrients improves, resulting in considerably improved performance. Waiting to wean calves until 8 weeks of age reduces the weaning performance gap often noticed in early-weaned calves (Eckert et al., 2015). When calves were weaned at 6 weeks, their metabolizable energy (**ME**) intake did not recover to pre-weaning intakes until 14 days after weaning (**Figure 3**). Calves weaned at 8 weeks, however, saw a recovery of ME intake to pre-weaning levels within 4 days. What these differing responses to the same dietary change demonstrate is the readiness of a calf's gut to adapt to a sudden change in the primary site of digestion and absorption.

Beyond intake, these data also show the immaturity of the nutrient absorption mechanisms. During the weaning transition, the ME:gain ratio, a measure of energy intake to body weight gain, was twice as high for calves weaned at 6 weeks (18.60 Mcal/kg) compared to calves weaned at 8 weeks (9.17 Mcal/kg; Eckert et al., 2015). These changes indicate that the 6-week-old calves were not only struggling with ME intake, but were also unable to effectively turn their feed intake into growth. Therefore, calves weaned too early are less effective at converting metabolizable energy intake into growth.
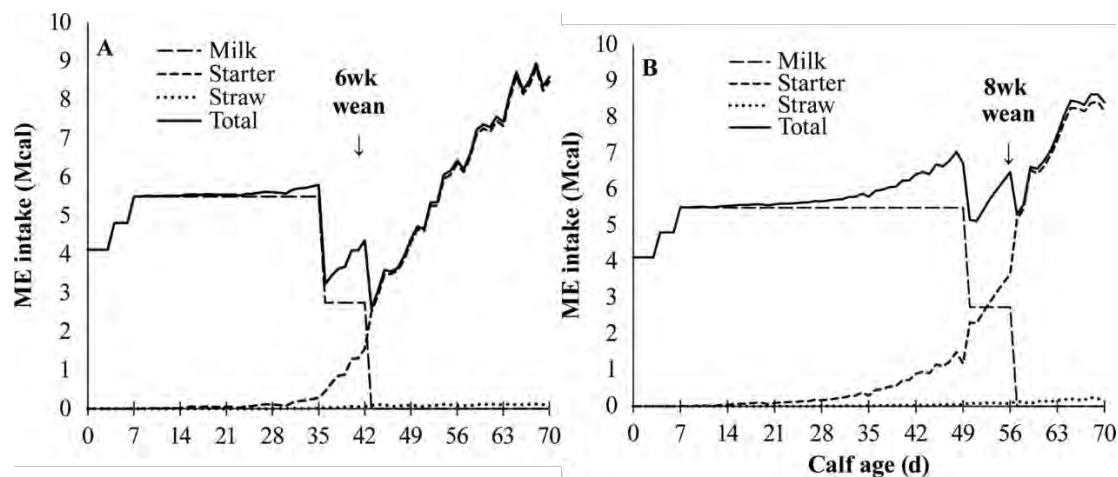
Figure 3. Change in metabolizable energy (ME) intake in calves weaned at 6 weeks (A) or 8 weeks (B). Calves weaned at 6 weeks showed a greater and longer drop in ME intake than calves weaned at 8 weeks. From Eckert et al. (2015).

Boosting Rumen Development Using Nutritional Stimuli

If calves are unable to turn their ME intake into growth, there is likely a compromised absorption ability. Little is known about how nutrient absorption mechanisms develop early in life and which factors promote them. Currently, the primary metric used to stimulate rumen development is the feeding of rapidly fermentable carbohydrates in calf starter. The impact of calf starter fermentation on rumen papillae development is clear (**Figure 1**) and well-documented in past research.

Fermentation of calf starter leads to production of butyrate, one of the VFA long linked to the development of rumen papillae (Warner et al., 1956; Figure 1). Butyrate is very bioactive, acting to alter gene expression of multiple genes involved in tissue adaptation (Baldwin et al., 2012). In adult cows, adding extra butyrate to the rumen improves VFA absorption capacity (Laarman et al., 2013a) and improves barrier function (Baldwin et al., 2012; Laarman et al., 2013b). Because of butyrate's diverse impact on rumen development and function, increasing butyrate production in young calves is a sought-after nutritional goal.

Several avenues exist for improving butyrate supply in the gut. Direct feeding of butyrate is the most familiar, as its inclusion in milk replacer and calf starter increases growth and rumen papillae development in week-old calves and prior to weaning (Gorka et al., 2018). When fed during the weaning transition to eight-week old calves, calf starter intake increases, and remains 800 g/d higher after butyrate supplementation is withdrawn (McCurdy et al., 2019). Interestingly, the impact of butyrate on rumen development is noticeable even when the butyrate is rumen-protected (Gorka et al., 2018; McCurdy et al., 2019). Through the stimulatory effects of butyrate, the rumen's nutrient transport mechanisms develop and ready the calf for weaning. Despite the clear impact of butyrate on morphological development and growth, its impact on nutrient absorption is far more nuanced, likely because of the complexity and plasticity of nutrient absorption dynamics.

Nutrient Absorption

Nutrient Transport Mechanisms

Nutrient absorption is a multi-factorial, multi-pathway system that takes place across every gastrointestinal epithelium. In young calves, the rumen epithelium is of principal interest, as it is non-functional. Currently, VFA absorption mechanisms are categorized primarily through transport pathways and transport proteins embedded in the epithelium that physically transport the VFA across the membrane. Transport pathways use kinetics measurements to determine the rate of VFA transport. Transport proteins, meanwhile, use abundance measurements to determine the capacity of VFA transport and potential targets for nutritional intervention to improve VFA transport.

Nutrient transport is accomplished through one of two mechanisms: passive diffusion or protein-mediated flux. Passive diffusion is the unregulated flow of VFA across the rumen epithelial membrane. For passive diffusion to occur, there needs to be a high VFA concentration in the rumen and a lower concentration in the epithelium, and the VFA need to be associated with a proton (**H-VFA**), as opposed to an anionic VFA (**VFA$^-$**). As rumen pH drops, H-VFA become more prevalent (**Table 1**), and there is more opportunity for H-VFA to freely diffuse across the lipid membrane of the epithelial cells.

Table 1. Prevalence of unregulated (H-VFA) and regulated (VFA$^-$) available for transport across the rumen wall at physiological pH (6.8), subacute ruminal acidotic pH (5.8), acute ruminal acidotic pH (5.2), and the pK$_a$ (4.8).

pH	H-VFA (Passive Diffusion)	VFA$^-$ (Protein-Mediated)
6.8	1 %	99 %
5.8	10 %	90 %
5.2	40 %	60 %
4.8	50 %	50 %

Several constraints limit the amount of passive diffusion. Firstly, the pK$_a$, the pH at which 50% of VFA are dissociated, is 4.8. Therefore, at physiological pH of 5.8 – 6.8, the abundance of H-VFA is below 10% of all VFA present in the rumen and does not provide an adequate avenue to meet the cow's energy needs. Even at acidotic pH (4.8 – 5.8), the abundance of H-VFA never exceeds 50%, so physiological rumen pH poses serious constraints on the ability of cows to effectively absorb ruminal VFA through passive diffusion alone. This is supported by research showing that the rate of VFA diffusion at decreasing pH does not match what would be predicted from biochemistry and lipophilicity of the VFA (Sehested et al., 1999). In a more recent study, a five-fold increase in VFA concentration resulted in only a 2.4-fold increase in VFA transport rates (Schurmann et al., 2014).

Part of the reason passive diffusion is more limited is because of the impact passive diffusion has on the epithelial cell. While rumen pH can vary from 6.8 to 5.2 without clinical incidence of ruminal acidosis (Aschenbach et al., 2011, Laarman et al., 2012b), the intracellular pH (**pH$_i$**) of epithelial cells is tightly regulated at 7.4, with programmed cell death occurring when pH$_i$ drops below 7.0 (Lagadic-Gossmann et al., 2004), leading to compromised barrier integrity.

When a H-VFA enters the cell via passive diffusion, its pK_a of 4.8 means it will immediately dissociate, adding a proton to the cell and thus lowering intracellular pH. The cell must then return pH_i to 7.4 by modifying H^+ and HCO_3^- in the cytosol. Although H-VFA may diffuse freely across the lipid membrane of the epithelium, H-VFA can ultimately only be transported completely across the epithelium via protein-mediated transport mechanisms.

Consequently, passive diffusion of VFA across the rumen epithelium is overshadowed by regulated, protein-mediated flux of VFA$^-$ (**Figure 4**). Protein-mediated VFA transport is mediated predominantly through bicarbonate-mediated transport, which comprises up to 50% of all VFA transport (Aschenbach et al., 2011). This involves transporters such as anion exchanger (**AE**), which export one HCO_3^- from the epithelial cell while importing one VFA$^-$ into the cell. There is also a bicarbonate-independent pathway, which is predominantly found in ruminants more efficient in VFA absorption, and consists of a nitrate-sensitive and nitrate-insensitive pathway (Penner et al., 2009). The bicarbonate-dependent and bicarbonate-independent comprise the two principal pathways of protein-mediated transport in the rumen.
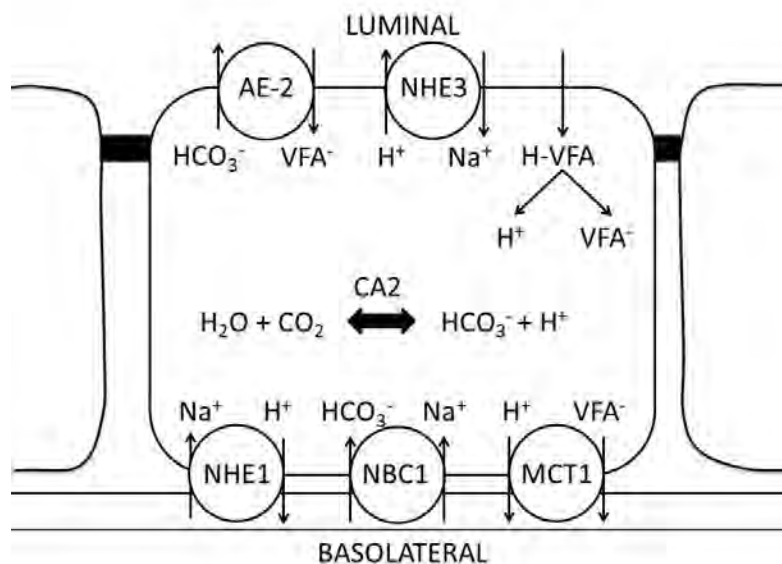
Figure 4. Principal VFA absorption mechanisms across the rumen epithelium; arrows indicate direction. Passive diffusion can occur at the luminal (rumen-facing) side, but H-VFA will dissociate upon entry into the epithelial cell (top right). Common transport-based mechanisms are shown, including bicarbonate-dependent mechanism (**AE2**), and a bicarbonate-independent mechanism (**MCT1**). Transport of VFA is intertwined with H^+ and HCO_3^- transport, therefore is intertwined with regulation of intracellular pH (**NHE1**, **NHE3**, **NBC1**). Not shown is a nitrate-sensitive transport pathway, the nature of which is not well-understood (Aschenbach et al., 2011). Schematic is simplified, merging strata of the rumen epithelium into one layer. From Laarman et al. (2015).

Development of Nutrient Transport Capacity

Currently, little is known about how the facilitated diffusion mechanisms develop in the calf. In the past, most research focused on the morphological and histological development of rumen papillae . Developed rumens, with greater papillae size and number, have a greater absorptive surface area, presumed to be indicative of VFA absorption capacity. Recently, however, the function of the epithelial cells was discovered to follow a trajectory quite different from

previously held assumptions. Furthermore, the nutrient transport pathways are regulated differently at different stages of early life.

Prior to weaning, calves fed milk and starter, as opposed to milk only, have greater expression of MCT1 mRNA (Laarman et al., 2012a), but not MCT1 proteins (Yohe et al., 2019; Hiltz et al., unpublished), or VFA clearance rates from the rumen (Yohe et al., 2019). Between abundance of mRNA, which code the instructions to build more proteins, and abundance of protein, which do the actual transport, there is clearly considerable regulation that is currently not well understood. Much potential lies in targeting these cellular processes to improve VFA transporter abundance in young calves.

During weaning, nutrient absorption capacity at the genomic level continues to change. At weaning, for instance, there is differential expression of over 20 genes involved in ion transport (e.g. VFA, H^+, HCO_3^-; Connor et al., 2013). Despite changes in mRNA of VFA transporters, there is no difference in protein abundance of VFA transporters during and after weaning (Hiltz et al., unpublished). Reasons for the disconnect between mRNA and protein likely relate to post-transcriptional regulation, an area that is poorly understood in food production animals. Nevertheless, the gastrointestinal tract can adapt considerably in early life.

Post-weaning, VFA absorption capacity remains adjustable, especially in response to dietary changes. In one study using seven-month-old crossbred weanling steers, dietary supplementation of a *Lactobacillus* fermentation product increased butyrate production *in vitro* and increased rates of gain *in vivo* (Hall et al., 2018). In another study, 7-month-old Holstein steers, feed restriction to 25% of their ad libitum intake resulted in increased passive diffusion of acetate and butyrate (Laarman et al., 2016). Throughout early life, plasticity in VFA absorption remains, suggesting much research remains to be done in developing optimal strategies for changing diets.

Integration of VFA Absorption and Intracellular pH

One constant theme in rumen epithelial adaptation is that adaptations in VFA transport are intertwined with adaptations in pH_i regulation. In a study on 7-month-old steers, VFA transport was positively correlated to pH_i regulation capacity (Laarman et al., 2016). In a different study, low pH without VFA did not damage rumen epithelia, while adding VFA to a low pH environment caused breakdown of the epithelium (Meissner et al., 2017). At low pH, the increase in passive diffusion of VFA put a downward pressure in pH_i, leading to cellular death and damage to the epithelium.

The link between VFA and pHi can also be found in the adaptation of the rumen epithelium to dietary changes. For instance, prior to weaning, the introduction of calf starter changes abundance of both NHE3 (Laarman et al., 2012a) and NBC1 (Hiltz et al., unpublished), both of which regulate pH_i. Similarly, during the weaning process, when diet fermentability increases dramatically, NHE3 expression changes and decreases pH_i regulation (Laarman and Oba, 2012a; Hiltz et al., unpublished). In adult cows, the lactation transition also exhibits decreased pH_i regulation (Laarman et al., 2015), suggesting the metabolic adaptation of the rumen epithelium is more centered around changes in pH_i regulation than direct VFA transport.

Despite nutrient absorption capacity historically being thought of as a function of absorptive surface area, research in the past decade showed definitively that nutrient absorption is closely linked to cellular physiology. The ability of epithelial cells to transport VFA and regulate their pH_i are intertwined, and are regulated at the mRNA, protein abundance, and protein activity

level. Throughout the first months of a calf's life, major changes in development are brought on by the consumption of rapidly fermentable solid feed and major dietary changes such as weaning. These major changes include not only changes in absorptive surface area, but considerable and consistent changes in pH_i regulation more so than changes in VFA absorption. Fostering improved nutrient absorption in young calves may therefore need to focus more on cellular physiology.

Nutrient Absorption & Health

Another reason why nutrient absorption is key to health is that the preferred energy substrate for the cells lining the gastrointestinal tract come from the lumen, not the blood. In the small intestine, the preferred energy substrates are the amino acids glutamine (Blachier et al., 2009), and, in the neonate, arginine (Wu et al., 2009). Unlike the small intestine, the large intestine and rumen use butyrate as a preferred energy substrate (Bergman, 1990). As a result, different preferred energy substrates are used for dietary supplementation in cows and calves. Another reason why nutrient absorption is key to health is the preferred energy substrate for the cells lining the gastrointestinal tract are sourced from the lumen, not the blood. In the small intestine, the preferred energy substrates are the amino acids glutamine (Blachier et al., 2009), and, in the neonate, arginine (Wu et al., 2009). Unlike the small intestine, the large intestine and rumen use butyrate as a preferred energy substrate (Bergman, 1990). As a result, preferred energy substrates are used for dietary supplementation in cows and calves.

Of the preferred energy substrates, the most abundant work involves butyrate supplementation. Used as rumen-protected supplement for calf starter, butyrate can have positive impacts on rumen development (Gorka et al., 2018) as well as starter intake (McCurdy et al., 2019), especially when fed around the weaning transition. Furthermore, butyrate is linked to decreased localized immune response (Dionissopoulos et al., 2013) and improved tight junction formation (Baldwin et al., 2012, Laarman et al., 2013b), indicating its ability to improve gastrointestinal health.

Likewise, glutamine is used to improve intestinal health. During the first months of life, glutamine and arginine are essential amino acids, playing major roles in intestinal energy supply and health (Wu et al., 2009, Wu et al., 2016). In calves, providing supplemental arginine or glutamine at 1% w/w in the milk replacer improved villus height, width, and surface area in the duodenum and jejunum when calves were fed a high plane of nutrition (van Keulen et al., 2020). Interestingly, the positive effects of glutamine or arginine supplementation are absent when calves are fed low amounts of milk replacer or a soy-protein concentrate (Drackley, 2008, Ahangarani et al., 2020, van Keulen et al., 2020). Although more research is needed on optimal supplementation strategies, conditionally essential amino acid supplementation can improve gut health in young calves.

Ultimately, supplementation strategies will only be successful if the preferred energy substrates can be absorbed by the intestine. Intestinal absorption of glutamate and glutamine requires facilitated transport (Howell et al., 2001), while absorption of butyrate is predominantly through facilitated transport (Sehested et al., 1999). Therefore, the health of gastrointestinal

epithelial cells is dependent on functional absorption capacity, leaving nutrient absorption and gut health very much intertwined.

Optimizing Nutrient Absorption and Health

Optimizing nutrient absorption and health in young calves are key for preparing calves for the health challenges common in early life, principally weaning and disease pressure. During weaning of 7-month-old beef calves, neutrophil counts increase and phagocytic function of the immune system decreases, suggesting impaired immune function (Lynch et al., 2010). Likewise, dairy calves often exhibit reduced circulating neutrophils and monocyte function during the weaning transition (Hulbert and Moisá, 2016). In both dairy and beef calves, weaning is a stressful event.

One of the principal ways of optimizing health during weaning is to ensure weaning occurs independent from other stressors. Oftentimes, weaning is concurrent with changes in housing (e.g., feedlot in beef, comingling in dairy), which can leave calves vulnerable to secondary disease pressures. In one study, beef calves that were weaned two weeks before transport to a new facility had a lower mortality rate from a secondary bacterial infection than calves that were weaned and transported on the same day (Hodgson et al., 2012). In another study, gradual weaning using a 2-week stepdown increased solid feed intake and growth post-weaning (Khan et al., 2007), highlighting the importance of stress management in increasing nutrient intake.

Through effective management strategies in early life, calves improve performance later in life. For instance, calves exposed to a *Salmonella enterica* Typhimurium challenge five weeks after weaning had improved immune response and 10% higher solid feed intake if they had been fed a high plane of nutrition prior to weaning (Ballou et al., 2015). In a different study(Ballou et al., 2015). Alternatively, dairy calves with greater liquid and solid feed intake prior to weaning produce more milk in their first lactation (Soberon et al., 2012, Rauba et al., 2019). In all, emphasizing nutrient intake and absorption as a principal goal early in a calf's life confers considerable health and productivity benefits in subsequent life stages.

Conclusion

Calves are born as pre-ruminants, with an underdeveloped and non-functioning rumen. In a mature rumen, nutrient digestion and absorption play critical roles in meeting the energy needs of the cow. Consequently, bolstering nutrient absorption capacity in young calves is an import goal of calf management strategies. Key to development of nutrient absorption capacity is ensuring a higher plane of nutrition to ensure growth requirements are met pre-weaning. Supplying a higher plane of nutrition will also directly benefit the cells lining the gastrointestinal tract. The gastrointestinal tract is fueled by energy substrates from the lumen, so having an adequate supply of butyrate and glutamine, arginine, and glutamate is essential for meeting the energy supply of the gastrointestinal epithelium.

In the rumen, feeding rapidly fermentable feeds such as calf starters will play an important role in developing the rumen epithelium to prepare it for the weaning transition. The development of the rumen epithelium involves changes in pH_i more so than changes in VFA transporters

directly. Furthermore, these transitions continue beyond weaning, so the freshly weaned calf is not yet a mature ruminant. In all, weaning imposes a major stress on the calf. Managed successfully, weaning should occur in isolation from other stressors like housing changes.

Together, both beef and dairy systems have similar goals for calf-raising programs. That is, the development of nutrient absorption capacity while maintaining a healthy gastrointestinal tract is one of the key concerns in preparing calves for weaning, regardless of age at weaning. Physiologically, weaning is a shift in the primary site of nutrient absorption from the small intestine to the rumen, which is inherently stressful. While cow/calf systems benefit from allowing calves more time on liquid feed before initiating weaning, dairy heifer systems benefit from aggressively pushing ruminal development prior to weaning through feeding rapidly fermentable calf starters. Through continued research into optimal feeding and absorption strategies, more effective calf raising strategies can be developed that further improve nutrient absorption capacity and health in young calves.

References

Ahangarani, M. A., A. Bach, A. Bassols, M. Vidal, D. Valent, S. Ruiz-Herrera, and M. Terré. 2020. Short communication: Performance, intestinal permeability, and metabolic profile of calves fed a milk replacer supplemented with glutamic acid. J. Dairy Sci. 103(1):433-438.

Allen, M. S. 1997. Relationship between fermentation acid production in the rumen and the requirement for physically effective fiber. J. Dairy Sci. 80(7):1447-1462.

Aschenbach, J. R., G. B. Penner, F. Stumpff, and G. Gabel. 2011. Ruminant Nutrition Symposium: Role of fermentation acid absorption in the regulation of ruminal pH. J. Anim. Sci. 89(4):1092-1107.

Baldwin, R. L. V., S. Wu, W. Li, C. Li, B. J. Bequette, and R. W. Li. 2012. Quantification of transcriptome responses of the rumen epithelium to butyrate infusion using RNA-seq technology. Gene Regul. Syst. Bio. 6:67-80.

Ballou, M., D. Hanson, C. Cobb, B. Obeidat, M. Sellers, A. Pepper-Yowell, J. Carroll, T. Earleywine, and S. Lawhon. 2015. Plane of nutrition influences the performance, innate leukocyte responses, and resistance to an oral Salmonella enterica serotype Typhimurium challenge in Jersey calves. J. Dairy Sci. 98(3):1972-1982.

Bergman, E. N. 1990. Energy contributions of volatile fatty-acids from the gastrointestinal tract in various species. Physiol. Rev. 70(2):567-590.

Blachier, F., C. Boutry, C. Bos, and D. Tomé. 2009. Metabolism and functions of l-glutamate in the epithelial cells of the small and large intestines. Am. J. Clin. Nutr. 90(3):814S-821S.

Connor, E. E., R. L. t. Baldwin, C. J. Li, R. W. Li, and H. Chung. 2013. Gene expression in bovine rumen epithelium during weaning identifies molecular regulators of rumen development and growth. Funct. Integr. Genomics 13(1):133-142.

de Passille, A. M., T. F. Borderas, and J. Rushen. 2011. Weaning age of calves fed a high milk allowance by automated feeders: Effects on feed, water, and energy intake, behavioral signs of hunger, and weight gains. J. Dairy Sci. 94(3):1401-1408.

Dionissopoulos, L., A. H. Laarman, O. AlZahal, S. L. Greenwood, M. A. Steele, J. C. Plaizier, J. C. Matthews, and B. W. McBride. 2013. Butyrate-mediated genomic changes involved in non-specific host defenses, matrix remodeling and the immune response in the rumen epithelium of cows afflicted with subacute ruminal acidosis. Am. J. Anim. Vet. Sci. 8(1):8-27.

Dirksen, G., H. G. Liebich, and W. Mayer. 1985. Adaptive changes of the ruminal mucosa and their function and clinical significance. Bovine Pr. 20:116-120.

Drackley, J. K. 2008. Calf nutrition from birth to breeding. Vet. Clin. North Am. Food Anim. Pract. 24(1):55-86.

Eckert, E., H. Brown, K. Leslie, T. DeVries, and M. Steele. 2015. Weaning age affects growth, feed intake, gastrointestinal development, and behavior in Holstein calves fed an elevated plane of nutrition during the preweaning stage. J. Dairy Sci. 98(9):6315-6326.

Girard, J., P. Ferre, J. Pegorier, and P. Duee. 1992. Adaptations of glucose and fatty acid metabolism during perinatal period and suckling-weaning transition. Physiol. Rev. 72(2):507-562.

Górka, P., Z. Kowalski, R. Zabielski, and P. Guilloteau. 2018. Invited review: use of butyrate to promote gastrointestinal tract development in calves. J. Dairy Sci. 101(6):4785-4800.

Hall, J. B., A. H. Laarman, M. K. Reynolds, and W. K. Smith. 2018. Performance of backgrounding steers fed diets containing monensin or a lactobacillus fermentation product. Transl. Anim. Sci. 2:S130-S133.

Hammon, H., J. Steinhoff-Wagner, U. Schönhusen, C. Metges, and J. Blum. 2012. Energy metabolism in the newborn farm animal with emphasis on the calf: endocrine changes and responses to milk-born and systemic hormones. Domest. Anim. Endocrinol. 43(2):171-185.

Hodgson, P. D., P. Aich, J. Stookey, Y. Popowych, A. Potter, L. Babiuk, and P. J. Griebel. 2012. Stress significantly increases mortality following a secondary bacterial respiratory infection. Vet. Res. 43(1):21.

Howell, J., A. Matthews, K. Swanson, D. Harmon, and J. Matthews. 2001. Molecular identification of high-affinity glutamate transporters in sheep and cattle forestomach, intestine, liver, kidney, and pancreas. J. Anim. Sci. 79(5):1329-1336.

Hulbert, L. E. and S. J. Moisá. 2016. Stress, immunity, and the management of calves. J. Dairy Sci. 99(4):3199-3216.

Khan, M. A., H. J. Lee, W. S. Lee, H. S. Kim, S. B. Kim, K. S. Ki, J. K. Ha, H. G. Lee, and Y. J. Choi. 2007. Pre- and postweaning performance of Holstein female calves fed milk through step-down and conventional methods. J. Dairy Sci. 90(2):876-885.

Laarman, A. H., L. Dionissopoulos, O. AlZahal, S. L. Greenwood, M. A. Steele, and B. W. McBride. 2013a. Butyrate and subacute ruminal acidosis affect abundance of membrane proteins involved with proton and short chain fatty acid transport in the rumen epithelium of dairy cows. Am. J. Anim. Vet. Sci. 8(4):220-229.

Laarman, A. H., L. Dionissopoulos, O. AlZahal, M. A. Steele, S. L. Greenwood, J. C. Matthews, and B. W. McBride. 2013b. Butyrate supplementation affects mRNA abundance of genes involved in glycolysis, oxidative phosphorylation and lipogenesis in the rumen epithelium of Holstein dairy cows. Am. J. Anim. Vet. Sci. 8(4):239-245.

Laarman, A. H., A. Kleinberg, M. A. Steele, O. AlZahal, and B. W. McBride. 2015. Changes in the rumen papillae during the periparturient transition in Holstein dairy cows are accompanied by changes in abundance of proteins involved in intracellular pH regulation, but not SCFA transport. Am. J. Anim. Vet. Sci. 10:14-22.

Laarman, A. H. and M. Oba. 2011. Short communication: Effect of calf starter on rumen pH of Holstein dairy calves at weaning. J. Dairy Sci. 94(11):5661-5664.

Laarman, A. H., R. A. Pederzolli, K. M. Wood, G. B. Penner, and B. W. McBride. 2016. Effects of subacute ruminal acidosis and low feed intake on short-chain fatty acid transporters and flux pathways in Holstein steers. J. Anim. Sci. 94(9):3729-3737.

Laarman, A. H., A. L. Ruiz-Sanchez, T. Sugino, L. L. Guan, and M. Oba. 2012a. Effects of feeding a calf starter on molecular adaptations in the ruminal epithelium and liver of Holstein dairy calves. J. Dairy Sci. 95(5):2585-2594.

Laarman, A. H., T. Sugino, and M. Oba. 2012b. Effects of starch content of calf starter on growth and rumen pH in Holstein calves during the weaning transition. J. Dairy Sci. 95(8):4478-4487.

Lagadic-Gossmann, D., L. Huc, and V. Lecureur. 2004. Alterations of intracellular pH homeostasis in apoptosis: origins and roles. Cell Death Differ. 11(9):953-961.

Lynch, E. M., B. Earley, M. McGee, and S. Doyle. 2010. Effect of abrupt weaning at housing on leukocyte distribution, functional activity of neutrophils, and acute phase protein response of beef calves. BMC veterinary research 6(1):39.

McCurdy, D., K. Wilkins, R. Hiltz, S. Moreland, K. Klanderman, and A. Laarman. 2019. Effects of supplemental butyrate and weaning on rumen fermentation in Holstein calves. J. Dairy Sci. 102:8874-8882.

Meissner, S., F. Hagen, C. Deiner, D. Günzel, G. Greco, Z. Shen, and J. R. Aschenbach. 2017. Key role of short-chain fatty acids in epithelial barrier failure during ruminal acidosis. J. Dairy Sci. 100(8):6662-6675. https://doi.org/https://doi.org/10.3168/jds.2016-12262

NRC. 2001. Nutrient requirements of dairy cattle. 7 ed. National Academy Press, Washington, DC.

Penner, G. B., J. R. Aschenbach, G. Gabel, R. Rackwitz, and M. Oba. 2009. Epithelial capacity for apical uptake of short chain fatty acids is a key determinant for intraruminal pH and the susceptibility to subacute ruminal acidosis in sheep. J. Nutr. 139(9):1714-1720.

Rauba, J., B. Heins, H. Chester-Jones, H. Diaz, D. Ziegler, J. Linn, and N. Broadwater. 2019. Relationships between protein and energy consumed from milk replacer and starter and calf growth and first-lactation production of Holstein dairy cows. J. Dairy Sci. 102(1):301-310.

Schurmann, B. L., M. E. Walpole, P. Gorka, J. C. Ching, M. E. Loewen, and G. B. Penner. 2014. Short-term adaptation of the ruminal epithelium involves abrupt changes in sodium and short-chain fatty acid transport. Am. J. Physiol. Regul. Integr. Comp. Physiol. 307(7):R802-R816.

Sehested, J., L. Diernaes, P. D. Moller, and E. Skadhauge. 1999. Ruminal transport and metabolism of short-chain fatty acids (SCFA) in vitro: Effect of SCFA chain length and pH. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 123(4):359-368.

Soberon, F., E. Raffrenato, R. Everett, and M. Van Amburgh. 2012. Preweaning milk replacer intake and effects on long-term productivity of dairy calves. J. Dairy Sci. 95(2):783-793.

Stamey, J. A., N. A. Janovick, A. F. Kertz, and J. K. Drackley. 2012. Influence of starter protein content on growth of dairy calves in an enhanced early nutrition program. J. Dairy Sci. 95(6):3327-3336.

Sweeney, B. C., J. Rushen, D. M. Weary, and A. M. de Passille. 2010. Duration of weaning, starter intake, and weight gain of dairy calves fed large amounts of milk. J. Dairy Sci. 93(1):148-152.

USDA. 2012. Dairy Heifer Raiser, 2011. USDA-APHIS-VS, ed.

van Keulen, P., M. A. Khan, J. Dijkstra, F. Knol, and S. A. McCoard. 2020. Effect of arginine or glutamine supplementation and milk feeding allowance on small intestine development in calves. J. Dairy Sci. *In press.*

Warner, R. G., W. P. Flatt, and J. K. Loosli. 1956. Ruminant nutrition, dietary factors influencing development of ruminant stomach. J. Agric. Food Chem. 4(9):788-792.

Wu, G., F. W. Bazer, T. A. Davis, S. W. Kim, P. Li, J. M. Rhoads, M. C. Satterfield, S. B. Smith, T. E. Spencer, and Y. Yin. 2009. Arginine metabolism and nutrition in growth, health and disease. Amino Acids 37(1):153-168.

Wu, M., H. Xiao, G. Liu, S. Chen, B. Tan, W. Ren, F. W. Bazer, G. Wu, and Y. Yin. 2016. Glutamine promotes intestinal SIgA secretion through intestinal microbiota and IL-13. Mol. Nutr. Food Res. 60(7):1637-1648.

Yohe, T., H. Schramm, R. White, M. Hanigan, C. Parsons, H. Tucker, B. Enger, N. Hardy, and K. Daniels. 2019. Form of calf diet and the rumen. II: Impact on volatile fatty acid absorption. J. Dairy Sci. 102:8502-8512.

Supporting healthy immune function in transition dairy cows

Soutenir le fonctionnement immunitaire durant la période de transition chez la vache laitière

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Abstract

There is a high risk of clinical or subclinical reproductive tract disease in the postpartum period in dairy cows. An integrated process of adaptive events should occur synchronously, including a robust but well-regulated immune response in the uterus. Failure of this process may result in reproductive tract inflammatory disease.

Neutrophils are the most abundant type of inflammatory cell and the main line of defence against infection in the uterus. Avoidance of clinical disease (metritis and purulent vaginal discharge) depends in large part on how effective the immune response is at limiting the burden and effects of bacterial pathogens, while the occurrence of subclinical endometritis is more a function of avoiding excessive or persistent inflammation.

Glucose supply, hypocalcemia, lipid mobilization from body fat, ketosis, and the flux of pro-inflammatory cytokines influence immune response and change rapidly and variably among individual cows. It is clear that hypocalcaemia is associated with reproductive tract disease, but the mechanisms remain unclear (i.e., direct effects of calcium on innate immune cell function vs. indirect effects of hypocalcemia through shared pathways related to feed intake or behaviour). Therefore, the importance of prevention or early treatment of subclinical hypocalcemia as a means to improve immune function and prevent reproductive tract disease is also unclear. Effective but well-regulated inflammatory response will be favoured by best management practices for transition cows, but specific interventions to modulate immune response to prevent uterine disease remain developmental.

Résumé

Le risque de maladie clinique ou subclinique de l'appareil reproducteur en postpartum est élevé chez les vaches laitières. Un processus intégré d'événements adaptatifs synchronisés, comprenant notamment une réponse immunitaire robuste mais bien régulée dans l'utérus, doit se déclencher. Le contraire peut entraîner une maladie inflammatoire de l'appareil reproducteur.

Les neutrophiles sont le type de cellules inflammatoires le plus abondant et la principale ligne de défense contre l'infection dans l'utérus. La prévention des maladies cliniques (métrite et pertes vaginales purulentes) repose en grande partie sur la capacité de la réponse immunitaire à limiter la charge et les effets des agents pathogènes bactériens, tandis que l'apparition d'une endométrite subclinique dépend plutôt de l'aptitude à prévenir l'inflammation excessive ou persistante.

L'apport en glucose, l'hypocalcémie, la mobilisation des lipides des graisses corporelles, l'acétonémie et le flux de cytokines pro-inflammatoires influencent la réponse immunitaire et changent rapidement et inégalement d'une vache à l'autre. Il ne fait aucun doute que l'hypocalcémie est associée aux maladies de l'appareil reproducteur, mais les mécanismes de cette association restent flous (c'est-à-dire les effets directs du calcium sur la fonction des cellules immunitaires innées par rapport aux effets indirects de l'hypocalcémie par des voies communes liées à la consommation d'aliments ou au comportement). Par conséquent, l'importance de la prévention ou du traitement précoce de l'hypocalcémie subclinique comme moyen d'améliorer la fonction immunitaire et de prévenir les maladies de l'appareil reproducteur n'est pas claire non plus. Une réponse inflammatoire efficace mais bien régulée sera favorisée par l'application des pratiques exemplaires de régie des vaches en transition, mais les interventions spécifiques visant à moduler la réponse immunitaire pour prévenir les maladies de l'utérus sont toujours en cours de développement.

Introduction

Dairy cows experience massive metabolic demands to support lactation. They adapt their metabolism to do so, including uncoupling of the somatotropic axis (Baumgard et al., 2017) with peripheral insulin resistance and increased lipolysis to fuel milk production. However, cows are challenged by a transient decrease in feed intake resulting in negative energy and protein balance in early lactation, and short-duration but substantial hypocalcemia around calving. Concurrently, there is impairment of innate immune function and regulation of inflammation which is central to the development of mastitis (Ballou et al., 2012) and uterine diseases (Sheldon et al., 2019). The mechanisms linking these changes and metabolic challenges are only partially understood.

Markers of aspects of adaptation to negative energy balance (e.g., serum concentrations of non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHB)) are associated with the risk of many metabolic and infectious diseases, in part through their associations with suppressed immune function and excessive inflammation (Ingvartsen and Moyes, 2013). Approximately 35% of peripartum cows have NEFA and 45% have BHB above thresholds associated with metabolic disease or compromised production or reproduction (McArt et al., 2013). In a large dataset, 44% of cows had at least 1 disease condition in early lactation, and of these, 39% had 2 or more separate diseases (Santos et al., 2010). Impaired innate immune function appears to have an important place in this web of metabolic health and disease.

This paper provides a brief narrative review of selected important determinants of health in dairy cows in the transition period around calving. Specifically, the focus is on management and social stressors, markers of adaptation to negative energy balance, and hypocalcemia, and their associations with neutrophil function.

General neutrophil function

Neutrophils marginate, and adhere to and 'crawl' along endothelial cells, then perform diapedesis to move from blood vessels into tissue (Kolackowska and Kubes, 2013). Once in tissues, neutrophils interact with damaged cells or bacteria to remove foreign cells through a variety of mechanisms (Figure 1) including phagocytosis and intracellular digestion by oxidation (e.g., oxidative burst in lysosomes), extra-cellular release of oxidants from neutrophil granules (e.g., myeloperoxidase; Figure 1), or casting neutrophil extra-cellular traps (NET's) of DNA (Nauseef and Borregaard, 2014; Liew and Kubes, 2019).
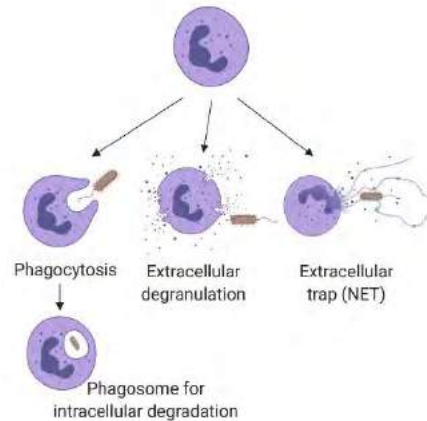
Figure 1. Schematic summary of the mechanisms by which neutrophils kill pathogens, such as bacteria as illustrated here. Neutrophil granules contain pro-inflammatory proteins including myeloperoxidase, lactoferrin, gelatinase, and matrix metalloproteinase 9. Following phagocytosis, encapsulated pathogens are killed intracellularly by reactive oxygen species or proteins from granules that fuse with the phagosome. Neutrophil extracellular traps consist of extruded DNA and cargo from extracellular granules.

It is now recognized that there are important links of systemic metabolism (especially adipose metabolism and insulin resistance) with immune function and inflammation in humans and laboratory animals (Hotamisligil and Erbay, 2006; Osborn and Olefsky, 2012) as well as in dairy cows (Bradford et al., 2015). Both obese people and periparturient dairy cows are characterized by elevated circulating NEFA, insulin resistance, hepatic lipid accumulation and systemic inflammation, and there is great interest in the phenomenon of metabolic or sterile inflammation in dairy cows (LeBlanc, 2014). NEFA, particularly saturated fatty acids that predominate in transition cows, may also impair neutrophil function (Ingvartsen and Moyes, 2013). Briefly, certain NEFA activate Toll-like receptor 4 (TLR4), a main receptor for lipopolysaccharide (LPS), which activates Nuclear Factor $\kappa\beta$ (NF-$\kappa\beta$)

and leads to secretion of Tumour Necrosis Factor α (TNFα), Interleukin 1 (IL-1) and IL-8. TNFα and IL-1 act on intracellular messengers to up-regulate inflammation and increase insulin resistance. Based on work in mice and humans, local, classical neutrophil actions may feed into chronic, systemic, sterile inflammation (Buck et al., 2017) under conditions that may include lipolysis, ketosis, and lack of supply of substrates for immune cells – all of which are common in dairy cows in the transition period. Host microbiome(s) are starting to be recognized as having important interactions with regulation of inflammation well beyond the gut (Kubes, 2018). However, there are also important differences between cows in early lactation and people with 'metabolic syndrome' or non-alcoholic fatty liver disease.

Neutrophil function in dairy cows

Classic work by Kehrli et al. (1989) demonstrated impairment of elements of innate immune function around calving, although the temporal changes may have been confounded to some extent by clinical or subclinical mastitis in 5 of the 8 primiparous animals studied. Most measures of function in neutrophils (except random migration the week before) were increased in the weeks preceding calving, followed by a nadir in week 1 after calving, particularly for their 3 measures of oxidative burst function. Each of retained placenta, metritis, purulent vaginal discharge, endometritis, and mastitis (which collectively affect at least 25% of dairy cows in early lactation) is strongly associated with impairments of one or more aspects of neutrophil function (Gunnink 1984; Cai et al., 1994; Kimura et al., 2002; Hammon et al., 2006; Ballou et al., 2012). While some reports document differences in neutrophil migration capacity between cows that subsequently have disease or remain healthy, the bulk of evidence points to impairment of neutrophil killing function (i.e., reduction of measures of oxidative burst such as iodination (myeloperoxidase activity), or cytochrome C reduction (generation of superoxide anion)). There is less indication of impairment of ingestion (phagocytic capacity) by neutrophils. The changes and differences in neutrophil function precede detection of clinical disease (Hammon et al., 2006) and may precede calving (Gunnink 1984; Cai et al., 1994; Kimura et al., 2002).

It is important to be specific when discussing neutrophil function, because not all aspects are typically impaired in transition cows, and different variables affect particular elements of neutrophil function.

In the transition period, there are inevitable substantial changes in circulating hormone concentrations in late pregnancy and the postpartum period and numerous profound endocrine adaptations to support lactation. These are compounded by imposed changes applied with good intentions but not always good effect (such as diet and pen or social group changes), as well as variable changes that may result from the foregoing or be somewhat independent of it, such as the degree of reduction in feed intake. Finally, other variables such as heat stress, competition for feeding or lying space, the quality of resting places, feed and water quality and availability, and the extent of social turmoil may combine to abate or exacerbate the inevitable challenges of the periparturient period.

In a bold experiment, researchers at the US National Animal Disease Center attempted to separate the effects of late pregnancy and calving from those of lactation by mastectomizing 10 cows in early to mid-pregnancy and comparing 3 markers of innate immune function to 8 intact cows that calved and lactated (Kimura et al., 1999). A caveat is that the effects of lactation may be confounded by the fact that all intact cows had milk fever and 3 of the 8 had ketosis and displaced abomasum. Expression of

L-selectin on the surface of neutrophils (necessary for initial (rolling) endothelial adhesion) decreased in both groups at calving but recovered within 1 to 3 days. The decrease was likely caused by inhibition of L-selectin (CD62) by glucocorticoids (Burton et al., 1995) i.e., cortisol released as part of parturition. Neutrophil surface expression of ☐2-integrins (needed for final adhesion for diapedesis) was actually greater before calving and to 3 d postpartum in the cows that lactated. However, myeloperoxidase activity (a measure of oxidative burst capacity) declined in both groups from 3 weeks before to 3 days after calving, but then quickly and fully recovered in the mastectomized cows while remaining at the parturient trough level until the end of the study at 21 d postpartum in the lactating cows. On the available evidence, oxidative burst is the most consistently impaired element of neutrophil function in dairy cows after calving, and these unique data support the inference that factors related to the demands of lactation sustain but do not initiate this impairment.

Factors affecting neutrophil function in dairy cows

There are numerous factors that are likely to affect neutrophil functional capacity in dairy cows, the headlines of which are illustrated in Figure 2. Elaboration is provided below.
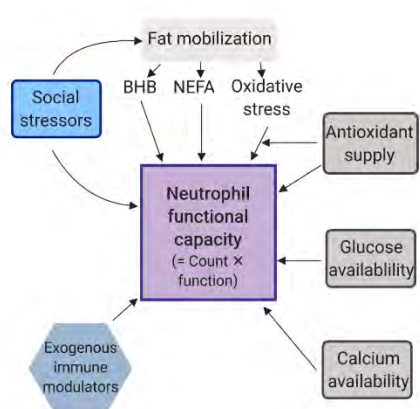
Figure 2. Factors that influence the functional response capacity of neutrophils in dairy cows. The scheme is simplified because there are likely interactions among these known factors, and others including genetics. Nutritional formulation and feeding management determine the potential supply of immune system inputs, with additional variability imposed by social group and competitive pressures, as well as heat stress, and the comfort of the lying space.

Management and social stress effects
Lesser feed intake up to 2 weeks before calving is a risk factor for metritis (Hammon et al., 2006; Huzzey et al., 2007). That finding makes sense because lower nutrient intake would plausibly reduce the availability of fuel and substrates for neutrophil function and so diminish immune response, which in turn is a risk factor for metritis (Cai et al., 1944; Hammon et al, 2006). The reasons for which some cows consume less than others before calving and before the onset of visible disease are not well understood. In the study by Huzzey et al. (2007), competitive behaviour at the feed bin was studied,

and cows that were more socially submissive (had fewer interactions in which they displaced another cow for feed access) were more likely to have lower intake and metritis. That study showed that cows apparently have a strong drive to eat at the same time, most acutely after fresh feed delivery, and that more submissive animals do not compensate their intake by eating at off-peak times. That is consistent with empirical observations that crowding or competition for space at the feed bunk (i.e., < 75 cm of feed bunk per cow or > 80% cows to headlocks) during the transition period is a risk factor for postpartum disease (Nordlund et al., 2006). Observations of the adverse effects on feed intake and lying time of pen moves and social group changes led to the logical suggestion that stable social groups and fewer movements of cows to new pens during the transition period (Cook and Nordlund, 2004), along with adequate feeding space, should reduce the incidence of postpartum disease. That ought to reduce stressors and/or improving feed intake for cows at the submissive end of the social spectrum, and so improve metabolic status and thereby immune function. Endogenous cortisol has a transient but substantial increase at calving, which is associated with decreased expression of glucocorticoid receptor on neutrophils and increased circulating neutrophil counts (Preisler et al., 2000). Both elevated endogenous cortisol concentrations and administration of dexamethasone decreased expression of L-selectin on neutrophils, leading to neutrophilia but likely contributing to decreased functional capacity of the neutrophil system for 1 to 2 days (Weber et al., 2004).

The hypothesis of social stress (mediated by acute or chronic elevations of cortisol or catecholamines and/or effects on feed intake or behaviour) leading to impaired immune function or health has generally not been supported in controlled studies. Huzzey et al. (2012) provided 4 groups of 10 cows in late gestation with 67 cm of feed rail space and 1 freestall per cow or 34 cm of feeding space and 2 cows per stall for 14 days per treatment in a crossover design. On average, feed intake was greater in the overstocked group, particularly in the second week of the treatment. There were significantly but modestly greater plasma NEFA and fecal cortisol metabolite concentrations, and lower plasma glucose concentrations in overcrowded primiparous animals, and a lesser insulin response to a glucose tolerance test in overcrowded cows. However, it is unclear that the magnitude of the effects observed would contribute to disease risk.

In an experiment, 756 Jersey cows were assigned 26 d before expected calving to separate pens for heifers or parous cows, stocked at either 80% cows to headlocks and 80% cows to freestalls, or approximately 100% cows to headlocks and stalls. There were more competitive displacements at the feed bunk in the crowded groups, but only small differences in mean feeding and lying times (Lobeck-Luchterhand et al., 2015). There were no differences between treatments in the incidence of retained placenta, metritis, purulent vaginal discharge, or culling, nor in plasma concentrations of NEFA or BHB, energy-corrected milk yield to 155 days of lactation, or probability of pregnancy at the first 2 inseminations (Silva et al., 2014). In a subset of 48 cows per treatment per parity, measures of innate and adaptive immune function and chronic stress were assessed from 2 to 3 weeks before, to 2 to 3 weeks after calving (Silva et al., 2016). There were no differences between treatments in neutrophil phagocytosis or oxidative burst function, expression of L-selection or cluster of differentiation (CD)18, production of immunoglobulin (Ig)G against ovalbumin, serum haptoglobin concentrations, or concentrations of cortisol in blood or hair.

To assess the effect of stable versus dynamic social group (pen populations) in the 4 weeks before calving, Silva et al. (2013a) assigned a total of 567 Jersey cows to groups of 44 cows per freestall pen in 6 replicates of stable ("all-in-all-out"; no additions to the pen) or dynamic (weekly additions of new

animals to replace cows that left for calving) social groups. That resulted in differences in social stability as well as average stocking density (72 and 87%, respectively, but with much greater variance in the stable groups). There were no differences between treatments in plasma concentrations of NEFA or BHB, the incidence of retained placenta, metritis, endometritis, lameness, or early culling, the prevalence of persistent anovulation, pregnancy at first or second insemination, or energy-corrected milk yield. In a subset of 34 to 40 cows per treatment, there were no differences between treatments in neutrophil phagocytosis or oxidative burst function or expression of L-selection or CD18, production of IgG against ovalbumin, serum hapoglobin concentrations, or concentrations of cortisol in blood (Silva et al., 2013b).

Miltenburg et al. (2018a) assigned 48 cows in groups of 6 to 10 to either of two space allowances for the 3 weeks before calving: 90 cm of feeding rail space per cow and 80% cows to freestalls, or 45 cm of feeding space and 120% cows to stalls. Lying time was reduced by 2 h/day in the overcrowded group but was still > 12 h/day on average. As seen by Silva et al. (2014), there were more competitive displacements at the feed bunk in overcrowded groups, although that was confounded with a greater number of such interactions among multiparous cows generally. Overcrowded cows tended to have greater liver fat content at week 3 postpartum. However, during the treatment period and to 5 weeks postpartum, there were no differences between treatments in serum BHB, NEFA, glucose, insulin, insulin-like growth factor 1, aspartate aminotransferase, bilirubin, or haptoglobin concentrations and no differences in neutrophil phagocytosis or oxidative burst function. One interesting finding was that when cows were ranked by successful displacements of other cows at the feed bunk, in under-crowded groups, medium and high success index animals had greater neutrophil oxidative burst function than low success cows or any cows in overcrowded groups. This suggests that greater space allowance may have an effect on innate immune function, but it is not to improve function for the most submissive cows. Similarly, Chebel et al. (2016) found that the cows in the 90th percentile of social rank (most dominant) had modestly but significantly greater neutrophil phagocytosis and oxidative burst capacity, particularly at calving. Also interestingly, and in contrast to Huzzey et al. (2007) compilation of data from several large experiments on space and social group stability indicated that, counter-intuitively, more dominant cows (based on displacements at the feed bunk) were at greater risk of retained placenta or metritis (Chebel et al., 2016). These authors hypothesize that such cows may spend more time in aggressive interactions but fail to use their apparent advantage to consume more feed or achieve greater health (at least with respect to uterine disease). We concluded from our study (Miltenburg et al., 2018a) and the published data on the effects of crowding on metabolic health and innate immune function that the evidence does not refute the potential importance of space allowances under field conditions, but feeding and lying space alone are not the critical determinants of immune function in transition dairy cows. The minimal and optimal amounts of feeding and lying space likely depend on other variables, including whether primiparous and multiparous animals are commingled in the prepartum period (Chebel et al., 2016)

Nutrient supply for neutrophil function
Mounting an immune or inflammatory response consumes meaningful quantities of nutrients, and is notably energetically costly. In an elegant experiment, Kvidera et al., (2017) employed an IV LPS challenge with a euglycemic clamp technique to estimate the minimal requirement of glucose to mount an acute inflammatory response, which was approximately a net of 1 kg of glucose in 12 h. The glucose requirement for immune response appears to be consistent among dairy and beef cattle at 0.7 to 1.0 g/kg body weight$^{0.75}$ per hour.

Homeorrhetic adaptions in support of lactation are oriented around partitioning nutrients to the mammary gland, notably increasing the supply of glucose for milk synthesis by sparing its use by other tissues, primarily by inducing peripheral insulin resistance. This situation likely contributes to reduction of immune function in early lactation. Activated immune cells appear to be obligate users of glucose and to increase their consumption of glucose (Kvidera et al., 2017). The fuels used by bovine neutrophils are not well characterized but glucose appears to be crucial (Ingvartsen and Moyes, 2013). Conversely, in vitro supplementation of glucose in neutrophils from early- or mid-lactation dairy cows modestly increased phagocytic capacity but mostly did not meaningfully affect PMN function (Garcia et al., 2015). However, the basal concentration of glucose in the cell media (7.2 mmol/L) used by Garcia et al. was considerably greater than in circulation in cows of either stage of lactation (~ 3.2 mmol/L), or even in dry cows. Once migrated, neutrophils likely must rely on stores of glycogen to function. Galvao et al. (2010) showed that there were lesser glycogen stores in neutrophils in circulation at calving in cows that 3 to 7 days later had metritis. That is consistent with Hammon et al. (2006), who showed that circulating neutrophils had lower oxidative burst capacity in cows that 1 week later had metritis, or 3 to 4 weeks later had endometritis. In that study, one explanatory variable was that neutrophils from cows in the lowest quartile of feed intake through the 3 weeks before calving (despite ad libitum availability) had (in relative terms) 50% lesser oxidative capacity from 1 week before to 3 weeks after calving than cows in the top quartile of feed intake. In summary, it seems that the availability of glucose to fuel neutrophil function may be a contributing factor to the impaired capacity observed in the transition period.

The supply of anti-oxidants (e.g., selenium and vitamin E) is important to contain the potent oxygen free radicals generated within neutrophils as part of their killing function. If there is insufficient selenium or sulfur-containing amino acids, glutathione peroxidase may not be able to detoxify hydrogen peroxide in the cytosol. Similarly, insufficient vitamin E may allow hydroxyl radicals to initiate a chain reaction of cell membrane peroxidation. Either could result in neutrophils conducting a short-lived suicide mission rather than a more sustained response with numerous iterations of ingestion and degradation of bacteria.

There is an abundance of large-scale observational studies that demonstrate associations of elevated serum concentrations of NEFA and/or BHB (hyperketonemia or ketosis) with increased risk of infectious and metabolic disease (summarized in McArt et al., 2013). Briefly, serum NEFA > 0.3 mmol/L in the 1 to 2 weeks before expected calving is associated with increased risk of retained placenta, metritis, or displaced abomasum, decreased milk production, and worse reproductive performance. Serum or blood BHB $\geq$ 1.2 mmol/L in the first 2 weeks after calving is associated with increased risk of displaced abomasum, endometritis, prolonged anovulation, and early culling, decreased milk yield in early lactation (conditional on the concentration of BHB and the timing of onset of ketosis), and decreased reproductive performance (McArt et al., 2013). Elevated serum NEFA concentrations and hyperketonemia are indicators of some degree of maladaptive response to the demands of lactation, probably in some measure reflect the availability of glucose to fuel neutrophils, and more generally are correlated with a greater degree of negative energy balance and perhaps heightened systemic inflammation, all of which are intertwined. In the whole animal, it is difficult to distinguish whether these markers are indictors of other complex processes, or NEFA or BHB might have direct effects on innate immune function. Several studies provide insight into this question, with inconsistent results.

NEFA

Neutrophils were collected from 8 Holstein cows in mid-lactation and incubated with mixtures of NEFA to resemble concentrations from low to typical in the days after calving (2-fold increments from 0.06 to 1 mmol/L) to extremely high (2 mmol/L) (Scalia et al., 2006). Phagocytic function was not affected at any concentration of NEFA, and oxidative burst function was only affected at 2 mmol/L, where it was substantially increased, but in conjunction with massive neutrophil necrosis (49% vs. < 1% at lower concentrations), though not increased apoptosis. It is unclear if brief in vitro exposure of circulating neutrophils to moderately elevated concentrations of NEFA replicates the direct or indirect effects of maturation and circulation in the milieu of a peripartum cow with elevated blood NEFA concentrations, which likely also includes increased concentrations of pro-inflammatory cytokines and other modulators of neutrophil function. Ster et al. (2012) also mixed neutrophils from mid-lactation cows with a mixture of NEFA at 0, 0.1, 0.25, 0.5, or 0.75 mmol/L to mimic concentrations in early postpartum cows. They observed a dose-dependent reduction in oxidative burst function, with significant and substantial impairment at the two higher concentrations. They did not measure other aspects of neutrophil function with the spiked NEFA approach, but they did demonstrate inhibition of proliferation of peripheral blood mononuclear cells (PMBC) with NEFA as low as 0.13 mmol/L. Hammon et al. (2006) found a significant but modest correlation between in vivo plasma NEFA concentration and neutrophil oxidative burst activity in vitro (R2 = 0.2). Taken together, these studies support a possible direct and rapid effect of elevated concentrations of NEFA on the functional capacity of circulating neutrophils, but encourage more investigation into the mechanisms by which perhaps specific fatty acids modulate neutrophil functions and whether such effects are more relevant during maturation or in circulation.

Ketosis

Greater severity of clinical mastitis has been observed in cows with ketosis (Kremer et al., 1993). Hoeben et al. (1997) isolated neutrophils from 7 high-producing (presumably non-ketotic) cows and employed several assays to assess oxidative burst function in samples with BHB added at 0.01, 0.05, 0.1, 1.0, or 2.5 mmol/L. They showed impairment of generation of hydrogen peroxide specifically, through not of superoxide anion or myeloperoxidase activity, with BHB $\geq$ 1.0 mmol/L. In an experiment with only 3 to 6 cows per group with naturally-occurring differences in blood BHB concentration, neutrophil chemotaxis was reduced in cows with BHB > 1.6 mmol/L (Suriyasathaporn et al., 1999). Neutrophils from these same cows were then incubated with different combinations of ketone bodies (BHB alone at 1.0 or 4.8. mmol/L; acetoacetate or acetone alone; or a combination of all three at high or low concentrations). BHB alone did not consistently impair chemotaxis, but the other ketones and the combination of all did in all cases. This experiment has the advantage of using neutrophils that had natural exposure to ketotic cows (presumably not only elevated BHB, although the cows were at 5 to 10 weeks in lactation, so not the complex metabolic and endocrine milieu of the transition period). Ster et al. (2006; details above) demonstrated that adding BHB up to 1 mmol/L had no effect on blood mononuclear cell proliferation or interferon-γ production, and up to 10 mmol/L (i.e., extremely high) had no effect on neutrophil oxidative burst function. Similarly, Hammon et al. (2006) found no association of blood BHB concentrations with neutrophil killing ability. As with NEFA, the effects of ketone bodies are inconsistent, but there is sufficient evidence to suggest a potential effect on migration and killing functions of neutrophils. It is unclear if BHB alone is a sufficient cause of impaired neutrophil function.

Calcium

Intracellular calcium signaling is a key element in the activation of neutrophils. Calcium acts as a second messenger for intracellular signal transduction for a variety of cell-surface receptors (Vig and Kinet, 2009). Neutrophils treated in vitro with EDTA, an extracellular calcium ion chelator, had severely reduced phagocytosis capacity (Ducusin et al., 2001). Therefore, hypocalcemia around calving may contribute to immune cell dysfunction. Neutrophils collected from cows with clinical milk fever had lower intracellular calcium concentrations and impaired phagocytosis compared to cows without parturient paresis (Ducusin et al., 2003). Kimura et al. (2006) isolated peripheral blood mononuclear cells (PMBC) from 27 cows through the transition period, 8 of which had developed clinical milk fever, and assessed the quantity and release of intracellular calcium stores. They showed that the stimulated flux of intracellular ionized calcium (iCa) was reduced at calving, and lower in cows that developed milk fever days from 12 days before the onset of clinical signs. Intracelluar iCa response of PMBC was low at the time of milk fever, but doubled following treatment with intravenous calcium, indicating prompt response of PMBC intracellular iCa to increased plasma concentrations of total calcium. The releasable intracellular iCa store decreased before calving and was correlated with blood calcium concentration and with intracellular iCa flux in response to stimulation. The authors suggested that intracellular stores of iCa may be diminished before calving as there is next efflux of iCa in an attempt to maintain blood calcium concentration, likely contributing to impaired PMBC function by decreasing the magnitude of intracellular iCa flux available to activate cell function.

Recent work demonstrates the calcium 'cost' of mounting an inflammatory response to an acute lipopolysaccharide (LPS) challenge. Using a model analogous to Kvidera et al. (2017) for glucose, Horst et al., (2018) showed that in the 12 h after challenge with LPS, blood calcium concentration was reduced by 32%, and maintenance of eucalcemia during that time required infusion of 12 g of Ca, or somewhat more than the typical deficit (8 to 10 g) in a cow recumbent with milk fever.

Subclinical hypocalcemia is highly prevalent among periparturient cows and is associated with increased risk of displaced abomasum (Chapinal et al., 2011) and milk production losses (Chapinal et al., 2012) and increased culling risk in early lactation (Roberts et al., 2012). Cows classified at high risk for metritis (having one or more of dystocia, twins, stillbirth or retained placenta) that were able to maintain serum calcium concentrations above 2.15 mmol/L had one-half and one-third the incidence of metritis and puerperal metritis, respectively, when compared to low metritis risk cows that were below this cut-point at least once in the first 3 days postpartum (Martinez et al., 2012). That study showed reduced total circulating neutrophil number, neutrophil phagocytosis and neutrophil oxidative burst capacity in cows with blood calcium < 2.15mmol/L through the first 3 days postpartum. Based on the variables measured in the study, at least two-thirds of the cases of metritis were estimated to be attributable to having blood calcium below 2.15 mmol/L in the first 3 days postpartum. Even if that is an over-estimate if more variables in more herds were considered, it points to sub-optimal calcemia contributing meaningfully to the occurrence of metritis, mediated at least in part by impairment of neutrophil function.

The same research group explored this association through experimental induction of hypocalcemia with a 24-hour infusion of an selective iCa chelator (Martinez et al., 2014). They used 10 mature, non-pregnant, non-lactating cows in a crossover design. By about 4 h after the start of treatment, steady-state plasma concentrations of ~0.75 mmol/L iCa and ~1.75 mmol/L total calcium were maintained

for 20 hours; therefore, the model replicated blood calcium levels in a hypocalemic (but not milk fever) cow in the day after calving. Feed intake (~ 5 vs. 10 kg DM/day), blood glucose (~4.2 vs 4.4 mmol/L), and insulin concentrations decreased and NEFA increased during treatment, so the effects of treatment may not all be directly attributable to calcium. Neutrophil phagocytosis and oxidative burst function decreased at the end of the infusion and continued to diverge negatively from the controls until 3 d after the end of the infusion. The data patterns for both measures of neutrophil function were similar to those in their field study (Martinez et al., 2012). Similar to Kimura et al (2006), experimentally-induced hypocalcemia decreased the stimulated intracellular iCa flux (Martinez et al., 2014). The data from the latter study support earlier experimental and observational data that transient (≤ 1 day) hypocalcemia contributes to impaired neutrophil function and consequently to disease risk.

Emerging data (McArt - see elsewhere in this issue) indicate that the pattern and duration of reduced blood calcium concentrations in the 4 days after calving are more predictive of disease risk and milk yield than the nadir concentration or single point measurements in the first 24 h after calving. This new approach to classifying hypocalcemia should be applied to study its effects on neutrophil function.

We evaluated (Miltenburg et al., 2018) whether administration of an injectable calcium supplement product at time of calving increased neutrophil oxidative burst or phagocytosis capacity. Cows (n = 27) from 4 farms were blocked by parity and randomly assigned to receive either a commercial injectable calcium supplement or a placebo within 12 hours after calving and again 24 hours later. In a separate study with the same protocol (Miltenburg et al., 2016), treatment increased serum total calcium at 24 hours postpartum, conditional on calcium concentration before treatment. Total serum calcium concentration (tCa), neutrophil oxidative burst and neutrophil phagocytosis capacity were measured from coccygeal blood samples before and 72 hours after the first treatment. The study animals were 23 first parity heifers and 6 multiparous cows. There was no effect of treatment on oxidative burst or phagocytosis. Therefore, despite plasma calcium concentration being associated with neutrophil function as described above, this study does not support the ability of supplemental calcium, as given to low-parity parturient cows soon after calving, to improve oxidative burst or phagocytosis capacity of neutrophils.

Martinez et al. (2018) used 80 cows in a 2 × 2 factorial experiment of positive or negative (-130 mEq/kg DM) DCAD with different dietary sources of vitamin D fed for 4 weeks before calving to assess a variety of health outcomes. The negative DCAD treatment increased plasma iCa and tCa at calving and 1 d later. Regarding neutrophil function, there were no effects of treatments on phagocytosis, and no interactions of the effects of DCAD and source of vitamin D on neutrophil function. Overall, cows fed calcidiol had better oxidative burst function postpartum than those fed cholecalciferol. Among multiparous cows, there was a modest effect of the negative DCAD diet to improve phagocytosis function before calving and oxidative burst function after calving. Therefore, improving maintenance of calcium homeostasis through dietary prevention approaches holds some promise for support of neutrophil function.

Conclusion

There is a body of evidence to support that each of glucose supply, blood concentrations of calcium, NEFA, and BHB are associated with the overall capacity of neutrophil responses in dairy cows in the

1 to 2 weeks after calving. Most commonly, the aspect of neutrophil function reported to be affected relates to oxidative burst, although that may be skewed because it is the most studied. There are fewer assessments of migration capacity, and still little in the bovine on the role of neutrophils in down-regulating inflammation after the initial response. Notably, none of the effects of these factors influencing neutrophil function has been consistent among studies. Furthermore, controlled studies of socially-competitive environments do not reproduce the effects on immune function or related clinical diseases that would be expected from empirical observations. These inconsistencies may be partially attributable to differences in the study populations or the methods of analysis of neutrophil function, but it seems more likely that the interactions of these known factors (and probably others) are the key determinants of effective innate immune function and inflammatory response. Future research should assess the interactions among markers of energy supply and metabolism, and of those with calcium supply, and investigate the effects of the timing and duration of these effects. It would also be particularly relevant in cattle to pursue the question of whether neutrophils behave or are regulated differently in infection, injury, and sterile inflammation (also known as metabolic inflammation). It would be of practical importance to understand whether glucose and calcium supply, or exposure to NEFA or BHB are critical during myelopoiesis, in circulation in blood, or both. The present state of scientific evidence is consistent with the notion that best management practices to support adaptive metabolism and prevent excessive negative energy balance or hypocalcemia should plausibly be beneficial for innate immune function in transition dairy cows. However, there are insufficient data to make specific recommendations that would consistently enhance neutrophil function or reduce the incidence of diseases understood to be consequences of impaired or dysregulated immune function.

References

Ballou MA 2012. Role in the etiology and pathophysiology of clinical mastitis in dairy cows. Journal of Animal Science 90,1466–1478

Baumgard LH, Collier RJ, and Bauman DE 2017. A 100-Year Review: Regulation of nutrient partitioning to support lactation. Journal of Dairy Science 100, 10353–10366.

Bradford BJ, Yuan K, Farney JK, Mamedova LK, and Carpenter AJ 2015. Invited review: Inflammation during the transition to lactation: New adventures with an old flame. Journal of Dairy Science 98, 6631-6650.

Burton JL, Kehrli ME, Kapil S, and Horst RL 1995. Regulation of L-selectin and CD18 on bovine neutrophils by glucocorticoids: effects of cortisol and dexamethasone. Journal of Leukocyte Biology 57,317–325.

Cai TQ, Weston PG, Lund LA, Brodie B, McKenna DJ, and Wagner WC 1994. Association between neutrophil functions and periparturient disorders in cows. American Journal of Veterinary Research 55, 934–943.

Chapinal NC, Carson ME, Duffield TF, Capel M, Godden S, Overton M, Santos JEP, LeBlanc SJ 2011. The association of serum metabolites with clinical disease during the transition period. Journal of Dairy Science 94, 4897–4903

Chapinal N, Carson ME, LeBlanc SJ, Leslie KE, Godden S, Capel M, Santos JEP, Overton MW, and Duffield TF 2012. The association of serum metabolites in the transition period with milk production and early lactation reproductive performance. Journal of Dairy Science 95, 1301–1309.

Chebel RC, Silva PRB, Endres MI, Ballou MA, and Luchterhand KL 2016. Social stressors and their effects on immunity and health of periparturient dairy cows. Journal of Dairy Science 99, 3217–3228.

Cook NV, and Nordlund KV 2004. Behavioral needs of the transition cow and considerations for special needs facility design. Veterinary Clinics of North America - Food Animal 20, 495–520.

Ducusin RJ., Sarashina T, Uzuka Y, Tanabe S, and Ohtani M 2001. Phagocytic response of bovine polymorphonuclear leukocytes to different incubation conditions and following exposure to some effectors of phagocytosis and different anticoagulants in vitro. Canadian Journal of Veterinary Research 65, 38-44.

Ducusin RJ, Uzuka Y, Satoh E, Otani M, Nishimura M, Tanabe S, and Sarashina T 2003. Effects of extracellular Ca2+ on phagocytosis and intracellular Ca2+ concentrations in polymorphonuclear leukocytes of postpartum dairy cows. Research in Veterinary Science 75, 27-32.
Galvão KN, Flaminio MJBF, Brittin SB, Sper R, Fraga M, Caixeta L, Ricci A, Guard CL, Butler WR, and Gilbert RO 2010. Association between uterine disease and indicators of neutrophil and systemic energy status in lactating Holstein cows. Journal of Dairy Science 93, 2926–2937.

Garcia M, Elsasser TH, Qu Y, Zhu X, and Moyes KM 2015. Glucose supplementation has minimal effects on blood neutrophil function and gene expression in vitro. Journal of Dairy Science 98, 6139–6150.

Gunnink JW. 1984. Pre-partum leucocytic activity and retained placenta. Veterinary Quarterly, 52–54.

Hammon DS, Evjen IM, Dhiman TR, Goff JP and Walters JL 2006. Neutrophil function and energy status in Holstein cows with uterine health disorders. Veterinary Immunology and Immunopathology 113, 21-29.

Hoeben D, Heyneman R, and Burvenich C 1997. Elevated levels of β-hydroxybutyric acid in periparturient cows and in vitro effect on respiratory burst activity of bovine neutrophils. Veterinary Immunology and Immunopathology 58, 165-170.

Horst EA, EJ Mayorga, M Al-Qaisi, MA Abeyta, SL Portner, CS McCarthy, BM Goetz, HA Ramirez-Ramirez, and LH Baumgard 2018. Effects of maintaining eucalcemia following immunoactivation in lactating cows. Journal of Dairy Science 101, Suppl. 2, 383. (abstr.)

Hotamisligil GS and Erbay E 2008. Nutrient sensing and inflammation in metabolic diseases. Nature Reviews Immunology 8, 923–934.

Huzzey JM, Veira DM, Weary DM, and von Keyserlingk MA 2007. Prepartum behavior and dry matter intake identify dairy cows at risk for metritis. Journal of Dairy Science 90, 3220–3233.

Huzzey JM, Nydam DV, Grant RJ, and Overton TR. 2012. The effects of overstocking Holstein dairy cattle during the dry period on cortisol secretion and energy metabolism. Journal of Dairy Science 95, 4421–4433.

Ingvartsen, KL and Moyes KM. 2013. Nutrition, immune function and health of dairy cattle. Animal 7, 112-122.

Kehrli ME, Nonnecke BJ, and Roth JA 1989. Alterations in bovine neutrophil function during the periparturient period. American Journal of Veterinary Research 50, 207-214.

Kimura K, Goff JP, and Kehrli ME 1999. Effects of the Presence of the Mammary Gland on Expression of Neutrophil Adhesion Molecules and Myeloperoxidase Activity in Periparturient Dairy Cows. Journal of Dairy Science 82, 2385–2392.

Kimura K, Goff JP, Kehrli ME, and Reinhardt TA 2002. Decreased neutrophil function as a cause of retained placenta in dairy cattle. Journal of Dairy Science 85, 544–550.
Kimura K, Reinhardt TA, and Goff JP 2006. Parturition and hypocalcemia blunts calcium signals in immune cells of dairy cattle. Journal of Dairy Science 89, 2588–2595.

Kolaczkowska E and Kubes P 2013. Neutrophil recruitment and function in health and inflammation. Nature Reviews Immunology 13, 159-175.

Kremer DJ, Burvenich C, Noordhuizen-Stassen EN, Grommers FJ, Schukken YH, Heeringa R, and Brand A 1993. Severity of experimental Escherichia coli mastitis in ketonemic and nonketonemic dairy cows. Journal of Dairy Science 76, 3428–3436.

Kvidera SK, Horst EA, Abuajamieh M, Mayorga MJ, Sanz Fernandez MV, and Baumgard LH 2017. Glucose requirements of an activated immune system in lactating Holstein cows. Journal of Dairy Science 100, 2360–2374.

LeBlanc SJ 2014. Reproductive tract inflammatory disease in postpartum dairy cows. Animal 8s, 54–63.

Liew PX, Kubes P. 2019. The neutrophil's role during health and disease. Physiological Reviews 99, 1223–1248.

Lobeck-Luchterhand KM, Silva PR, Chebel RC, and Endres MI 2015. Effect of stocking density on social, feeding, and lying behavior of prepartum dairy animals. Journal of Dairy Science 98, 240–249.

Martinez N, Risco CA, Lima FS, Bisinotto RS., Greco LF., Ribeiro ES, Maunsell F, Galvão K, Santos JEP 2012. Evaluation of peripartal calcium status, energetic profile, and neutrophil function in dairy cows at low or high risk of developing uterine disease. Journal of Dairy Science 95, 7158–7172

Martinez N, Sinedino LPD, Bisinotto RS, Ribeiro ES, Gomes GC, Lima FS, Greco LF, Risco CA, Galvão KN, Taylor-Rodriguez D, Driver JP, Thatcher WW, and Santos JEP 2014. Effect of induced subclinical hypocalcemia on physiological responses and neutrophil function in dairy cows. Journal of Dairy Science 97, 874–887.

Martinez N, RM Rodney, E Block, LL Hernandez, CD Nelson, IJ Lean, and JEP Santos 2018. Effects of prepartum dietary cation-anion difference and source of vitamin D in dairy cows: Health and reproductive responses. Journal of Dairy Science 101, 2563–2578.

McArt JAA, Nydam DV, Oetzel GR, Overton TR and Ospina PA 2013. Elevated non-esterified fatty acids and β-hydroxybutyrate and their association with transition dairy cow performance. The Veterinary Journal 198, 560-570.

Miltenburg, CL, Duffield TF, Bienzle D, Scholtz E, and LeBlanc SJ 2016. Randomized clinical trial of a calcium supplement for improvement of health in dairy cows in early lactation. Journal of Dairy Science 99, 6550–6562.
Miltenburg, CL, Duffield TF, Bienzle D, Scholtz E, and LeBlanc SJ 2018a. The effect of prepartum feeding and lying space on metabolic health and immune function. Journal of Dairy Science 101, 5294–5306.

Miltenburg, CL, Duffield TF, Bienzle D, Scholtz E, and LeBlanc SJ 2018b. Short communication: The effect of calcium supplementation at calving on neutrophil function. Journal of Dairy Science 101, 9505–9509.

Nauseef WM, and Borregaard N 2014. Neutrophils at work. Nature Immunology 15, 602-611.

Nordlund K, Cook N, and Oetzel G 2006. Commingling Dairy Cows: Pen Moves, Stocking Density and Health. Proceedings of the American Association of Bovine Practitioners 39, 36-42.

Osborn O and Olefsky JM 2012. The cellular and signalling networks linking the immune system and metabolism in disease. Nature Medicine 18, 363–374.

Preisler MT, Weber PS, Tempelman RJ, Erskine RJ, Hunt H, Burton JL. 2000. Glucocorticoid receptor down-regulation in neutrophils of periparturient cows. American Journal of Veterinary Research 61,14-19.

Santos JE, Bisinotto RS, Ribeiro ES, Lima FS, Greco LF, Staples CR and Thatcher WW. 2010. Applying nutrition and physiology to improve reproduction in dairy cattle. Society for Reproduction and Fertility Suppl. 67, 387-403.

Scalia D, Lacetera N, and Bernabucci U. 2006. In vitro effects of nonesterified fatty acids on bovine neutrophils oxidative burst and viability. Journal of Dairy Science 89, 147–154.

Sheldon, IM, Cronin JG, and Bromfield JJ 2019. Tolerance and Innate Immunity Shape the Development of Postpartum Uterine Disease and the Impact of Endometritis in Dairy Cattle. Annual Reviews in Animal Biosciences. 7, 11.1–11.24

Silva PRB, Moraes JGN, Mendonça LGD, Scanavez AA, Nakagawa G, Fetrow J, Endres MI, and Chebel RC 2013a. Effects of weekly regrouping of prepartum dairy cows on metabolic, health, reproductive, and productive parameters. Journal of Dairy Science 96, 4436–4446.

Silva PRB, Moraes JGN, Mendonça LGD, Scanavez AA, Nakagawa G, Ballou MA, Walcheck B, Haines D, Endres MI, and Chebel RC 2013b. Effects of weekly regrouping of prepartum dairy cows on innate immune response and antibody concentration. Journal of Dairy Science 96, 7649–7657.

Silva PR, Dresch AR, Machado KS, Moraes JG, Lobeck-Luchterhand K, Nishimura TK, Ferreira, MA, Endres MI, and Chebel RC 2014. Prepartum stocking density: effects on metabolic, health, reproductive, and productive responses. Journal of Dairy Science 97, 5521–5532.

Silva PRB, Lobeck-Luchterhand KM, Cerri RLA, Haines DM, Ballou MA, Endres MI, and Chebel RC 2016. Effects of prepartum stocking density on innate and adaptive leukocyte responses and serum and hair cortisol concentrations. Veterinary Immunology and Immunopathology 169, 39–46.

Ster C, Loiselle MC and Lacasse P 2012. Effect of postcalving serum nonesterified fatty acids concentration on the functionality of bovine immune cells. Journal of Dairy Science 95, 708–717.

Suriyasathaporn W, Daemen AJJM, Noordhuizen-Stassen EN, Dieleman SJ, Nielen M, Schukken YH 1999. β-hydroxybutyrate levels in peripheral blood and ketone bodies supplemented in culture media affect the in vitro chemotaxis of bovine leukocytes. Veterinary Immunology and Immunopathology 68, 177-186.

Weber PSD, Toelboell T, Chang L-C, Durrett Tirrell J, Saama PM, Smith GW, and Burton JL 2004. Mechanisms of glucocorticoid-induced down-regulation of neutrophil L-selectin in cattle: evidence for effects at the gene-expression level and primarily on blood neutrophils. Journal of Leukocyte Biology 75, 815–827.

Vig M. and Kinet JP 2009. Calcium signaling in immune cells. Nature Immunology 10, 21-27.

Feeding pigs and broilers for their health: the need to go beyond animal performance - from a meta-analytic approach to animal trials.

Alimenter les porcs et les poulets de chair pour leur santé: la nécessité d'aller au-delà des performances des animaux - d'une approche méta-analytique aux expérimentations animales.

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Abstract

Animal performance, carcass quality and net return have long been the gold standard of animal production, targets that are typically achieved through adaptation of nutritional strategies. We are, however, witnessing a paradigm shift based in current bans on antibiotics' use as growth promoters and as prophylactics worldwide. These challenges entail constantly adapting our practices and the development of ever more efficient animal performance predictors. This requires multicriteria quantitative tools, rather than "one size fits all" approach to respond to animal requirements allowing also the ability to adapt quickly to constant changes (e.g. genetics, regulations, ingredients variation). Given the growing accumulation of experimental data per topic of interest (e.g. increasing numbers of publications / topic and of measured data / publication), an efficient data utilization is by gathering previous research on a specific topic, using data mining techniques such as meta-analysis to generate new knowledge. This, combined with classic nutritional trials with the aim of keeping growth performance at genetic potential and animal health. Having poor growth performance with diet supposed to fulfil nutrient requirements can often be associated with health problems but the opposite is not that obvious. This review will show some insights of animal performance and health status obtained from meta-analysis and animal trials to develop multicriteria approach to (1) balance dietary calcium and phosphorus to optimize performance, improve litter quality and reduce footpad dermatitis (FPD), (2) to reduce crude protein to improve broilers health by reducing litter moisture, FPD and nitrogen excess leading to reduction in ammonia and uric acid, and (3) to understand better the effect of alternatives to antibiotics growth promoters.

Résumé

Les performances des animaux, la qualité des carcasses et la rentabilité sont depuis longtemps les références en matière de production animale, des objectifs qui sont généralement atteints par l'adaptation de stratégies nutritionnelles. Nous assistons toutefois à un changement de paradigme fondé sur l'interdiction actuelle de l'utilisation des antibiotiques comme stimulateurs de croissance et comme prophylactiques dans le monde entier. Ces défis supposent une adaptation constante de nos pratiques et l'élaboration de prédicteurs de performance animale toujours plus efficaces. Ainsi, pour répondre aux besoins des animaux, il nous faut des outils quantitatifs multicritères, plutôt qu'une approche de type « taille unique », qui permettent aussi de s'adapter rapidement aux changements continuels (par exemple, la génétique, la réglementation, la variabilité des ingrédients). Étant donné l'accumulation croissante de données expérimentales par sujet d'intérêt (par exemple, le nombre croissant de publications par sujet et de données mesurées par publication), une utilisation efficace des données consiste à rassembler les recherches antérieures sur un sujet spécifique et à utiliser des techniques d'exploration de données, telles que la méta-analyse, pour générer de nouvelles connaissances. Ce qui précède étant combiné avec des essais nutritionnels classiques dans le but de maintenir des performances de croissance correspondant au potentiel génétique ainsi que de préserver la santé des animaux. On associe souvent à des problèmes de santé de mauvaises performances de croissance obtenues avec un régime alimentaire censé répondre aux besoins en nutriments, mais le contraire n'est pas si évident. Cette étude présente un aperçu des performances et de l'état de santé des animaux obtenus à partir de méta-analyses et d'essais afin d'élaborer une approche multicritères permettant (1) d'équilibrer le calcium et le phosphore alimentaires pour optimiser les performances et la qualité de la litière, (2) de réduire la protéine brute pour réduire l'humidité des litières, la pododermatite et les excès d'azote (ammoniac et acide urique) et (3) de mieux comprendre les effets des solutions de remplacement aux antibiotiques facteurs de croissance.

Introduction

According to the FAO, livestock is one of the fastest growing sectors of global agricultural production. It is evolving in response to the rapid increase in demand for products of animal origin, especially in developing countries. In developed countries, through intensification practices that have helped increase yields and efficiency while bringing down costs, the demand for livestock products is stagnating and have to face many challenges, among them, animal wellbeing must be monitored with increased vigilance and the fair and sustainable use of antibiotics must be found. Also, we can expect that animal production will increasingly be affected by the food-feed competition and natural resources as well as evolving in a carbon-constrained conditions. Finally, farmers must use excreta in ways that do not pose a threat to human health or cause blooms of blue-green algae and threaten ecosystem biodiversity.

Besides, modern farms and agricultural operations work far differently than those a few decades ago, primarily because of advancements in technology. It can be expected that these advanced devices and the precision they achieve, will improve sustainability by increasing efficiency, safety, with lower environmental footprint (Flachowsky and Kamphues, 2012). To be available and used accurately in the field, technology should go beyond mere tool making, and address how we

process valuable data. With all these constraints and changes, multi-criteria approach become necessary to produce sustainable animal protein. In that context, meta-analysis and mechanistic modeling that allow identifying trends and outcomes in multiple studies to draw conclusions based on results of a wide range of experiments rather than single experiments, thereby increasing reliability, are tools all indicated.

The current review will show some insights of animal performance and health status obtained from synergy between meta-analysis and animal trials to develop multicriteria approach to manage growth performance and animal health by (1) founding the between balance dietary calcium (Ca) and phosphorus (P), (2) assess the possibility of lowering dietary crude protein (LCP), and (3) to find alternatives to antibiotics growth promoters.

1. Meta-analysis of experimental data, a knowledge generator tool

Meta-analysis constitutes a scientific approach which carries out critical reviews and statistical studies based on previous research results in order to improve and empirically quantify knowledge on a subject. In animal science, meta-analysis has proven to be an efficient way to renew already published data to return to the user by creating new empirical models allowing to progress in both understanding and prediction aspects (St-Pierre, 2001; Sauvant et al., 2005, 2008). The progress is allowed by (1) the reduction of all biases and imprecision and, (2) by enlarging a priori the domain of validity of the model. This methodology has improved over time with several published meta-analyses in animal science increases with a rate of about 15% stressing an actual success (Sauvant et al., 2019). Meta-analysis is a time-consuming exercise that should ideally be done by specialist in the subject under study.

The main steps are the definition of the research question that allow the identification of keywords for the exhaustive publication search, the selection of publications, the coding and construction of the database, the graphical examination of the meta-design, the choice of the statistical model and the post-analyses (Figure 1). Note that this is an iterative process and it is not uncommon to return to the previous steps during the analysis.
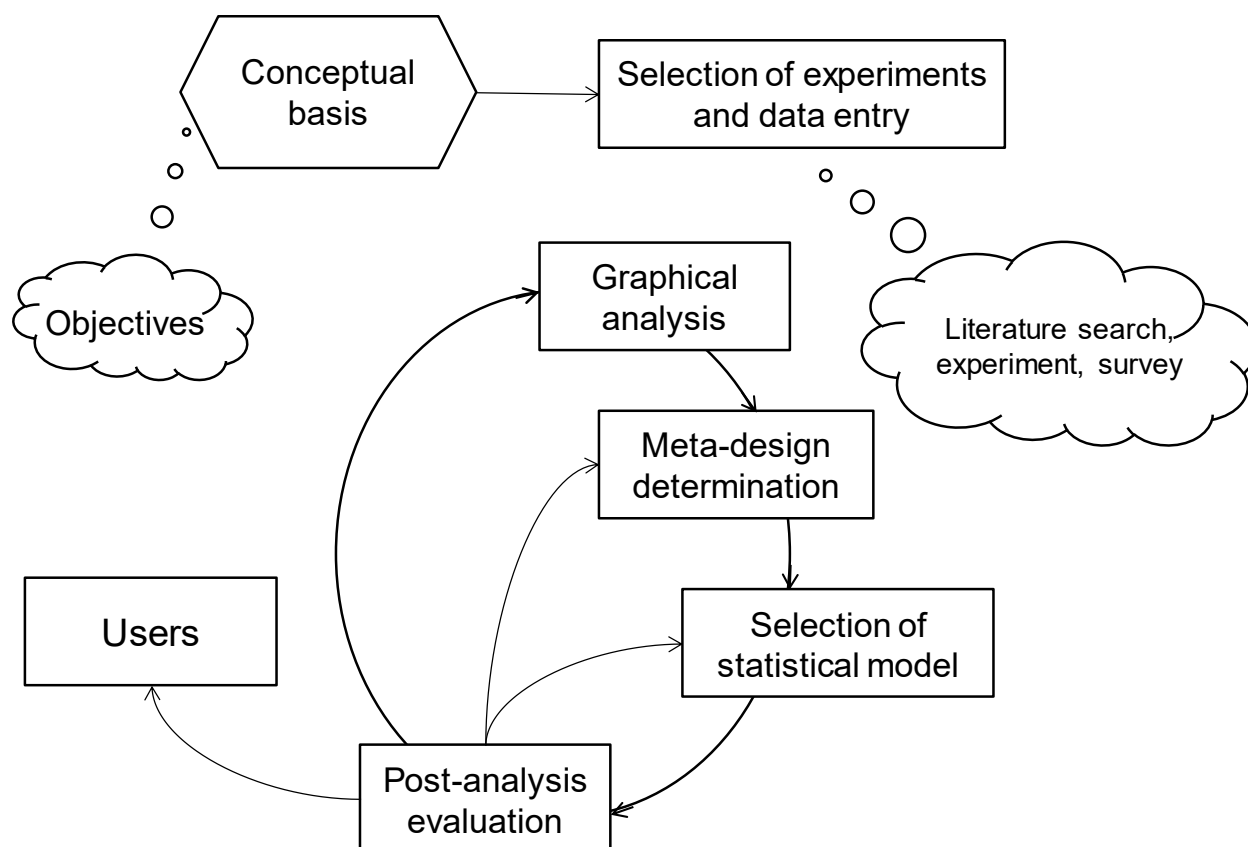
Figure 1. Meta-analysis steps (Sauvant et al., 2005)

A meta-analysis can be performed for different purposes, namely to (1) built mechanistic models (e.g. Létourneau-Montminy et al., 2015), (2) empirical laws of response $\Delta Y = f(\Delta X)$, for example of a causal feeding practice quantified by ΔX, which has been studied in a set of experiments, (3) increase the statistical power of an effect, (4) raise a doubt on the discordant results, (5) test and increase the possibilities of generalization, (6) explain the variability of the results, and (7) answer a question not asked in the tests. In animal science, meta-analysis are essentially interested in relations between variables and are mainly aimed at predicting the average quantitative response (ΔY), within the experiment, of a character Y with one or more X quantitative explanatory/predicting characters treated as independent covariables [i.e. $\Delta Y = f(\Delta X)$]. Under these conditions, the experiment effect corresponds to the variations between studies not considered by the covariables (Sauvant et al., 2019).

2. Meta-analysis as a tool to generate empirical relationships

Finding the right amount and balance between dietary calcium and phosphorus
In the new millennium, many countries including Canada have been under tremendous pressure to address environmental concerns with legislation aimed at reducing nitrogen (N) and phosphorus (P) excretion, including moratoriums in some provinces such as Quebec. Animal manure nevertheless remains valuable as fertilizer when manage correctly and can replace chemical

fertilizers successfully see more advantageously regarding soils structure. A better understanding of the fate of dietary P use by animals is necessary to optimize its use and enhance sustainable practices. The optimization of P utilization is complicated by the multiple criteria, such as growth performance, bone mineralization, and manure P used for assessment of requirements. This is further complicated by Ca concentration which is an important factor in the variability of P utilization by animals. In fact, first as constituents of skeletal hydroxyapatite and second through common hormonal regulation pathways, P and Ca are closely related. Besides, excessive intake of Ca can reduce P absorption and exacerbate symptoms of P deficiency (Létourneau-Montminy et al., 2012; Létourneau-Montminy et Narcy, 2013) by forming insoluble complexes in the gastrointestinal tract (Narcy et al., 2013; Heaney and Nordin, 2002) and thereby reducing efficiency (Suttle, 2010). Therefore, meta-analysis has been performed in pigs (Létourneau-Montminy et al., 2012, 2013) and broilers (Létourneau-Montminy et al., 2010; Couture et al., 2019a; Hedli et al., 2019) with many objectives among them, (1) to evaluate the impact of non-phytate P (NPP), phytate-P (PP), Ca and phytases on digestible P, retained P, bone ash, and growth performance by **generating empirical equations** and (2) to study the impact of Ca on phytase efficacy where their was **confounding results in the literature**.

Using apparent total tract digestibility data in pigs (Nexp = 86; Ntrt = 377) and ileal digestibility data in broilers (Nexp = 95; Ntrt = 480), a linear relationship between NPP and digestible P was established in both pig and broiler, so it is likely that P transport across the digestive wall did not represent a limiting step for P absorption, at least in the conditions of the experiments introduced in the database. Both NPP from mineral, animal and plant origin are highly digestible (from 56 to 70% in broilers and from 73 to 78% in pigs).

Regarding PP, large difference was observed between species with 21% of PP available without phytase in pigs as compared with more than 60% in broilers in which the digestibility of PP is lower in high Ca diets (Ca × PP, P<0.001) which was not the case in pigs (No interaction). The previous findings are consistent with results showing that broilers presented intestinal endogenous phytase activity depending of Ca (Applegate et al., 2003) and P supply (Valable et al., 2017). The digestibility of NPP is also reduced in both species by high Ca diets (Ca ×NPP). Likewise, in high-Ca diets, the unabsorbed dietary Ca would interact with P in the chyme to form insoluble salts, resulting in reduced absorption of dietary P (Heaney and Nordin, 2002).

Meta-analysis has showed that this reduction in digestibility in high Ca and low NPP diets induces reduction of feed intake, gain and feed conversion ratio in both pigs and broilers. The impact on feed conversion ratio needs more research to understand why the decrease of gain is higher than that of feed intake. Besides, given these two minerals are deposited together into bone, in a ratio of 2.2:1 to form a hydroxyapatite-like ($Ca_{10}(PO_4)_6(OH)_2$) compound (Crenshaw, 2001), unless the decrease in dietary Ca concentration could ameliorate P digestibility, it may cause an imbalance between Ca and P that leads to extra urinary losses of P and impairs P retention (De Rauglaudre et al., 2020). In fact, increasing dietary Ca negatively affects both retained P and growth performance in low-NPP diets but increases retained P and does not influence growth performance in high-NPP diets (Figure 2; Létourneau-Montminy et al., 2012).
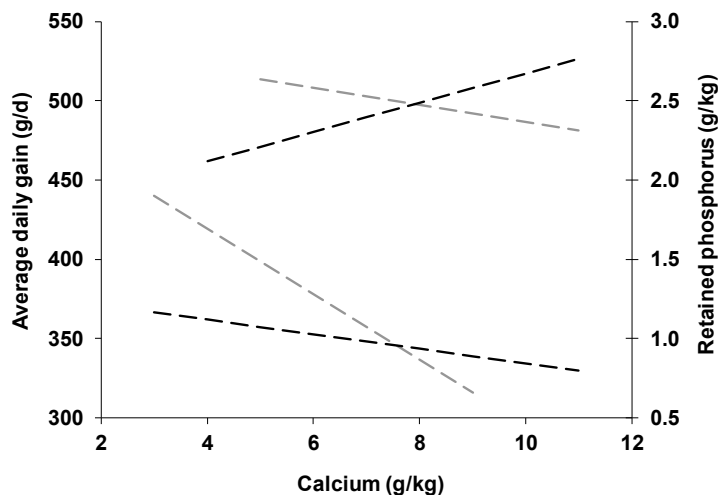
Figure 2. Responses of retained phosphorus (g/kg) and average daily gain (g/day) to dietary concentrations of non-phytate P (NPP, g/kg) and calcium (Ca, g/kg) with phytate P (PP) 2.2 g/kg, microbial phytase (PhytM) 0 FTU/kg and plant phytase (PhytP) 0 FTU/kg. The black and gray lines represent retained phosphorus and average daily gain, respectively. The solid and dotted lines represent 1.1 and 2.1 g NPP/kg, respectively.

Regarding microbial phytase, at the time we did the meta-analysis, it was stated that feeding poultry or swine with wide Ca:P ratios decreases the efficacy of phytase, and therefore lower ratio were recommended when using phytase (e.g. Qian et al., 1996; Kornegay et al, 1996; Sebastian et al., 1996). However, examination of the data indicates that a significant portion of the literature published over the 90s concerning the efficacy of phytase at different dietary Ca and P ratios is misleading because these references do not make the distinction between the concentrations of Ca and P in which phytase results in maximum animal performance and the concentrations of Ca and P in which phytase is most efficient in liberating P. The **doubt has been raised** by 2 meta-analysis works in broilers showing that the effect of phytase is more marked in high Ca and low P diet in terms of precaecal P digestibility, growth performance and bone ash in broilers (Létourneau-Montminy et al., 2010, 2012; Couture et al., 2019a) while in pigs, there was no effect of Ca on phytase efficacy in liberating P. Nevertheless, in pig an increase in P urinary losses will occur if phytase is added in a low Ca diet (Narcy et al., 2012).

In sum, with the meta-analysis tool it has been showed that Ca did not reduce the efficacy of phytase in liberating P in both species. The equations predicting apparent total tract digestibility in pigs and precaecal digestibility in broilers were **integrated into mechanistic models** (Létourneau-Montminy et al., 2015; Couture et al., 2019b) to predict absorption.

Although powerful, **meta-analysis is limited by the data available**. Health status in broilers is closely associated with litter quality (e.g. litter moisture). In this connection, Ca and P homeostasis is achieved through physiological mechanisms involving osmoregulation, suggesting the indirect role of these minerals with litter quality. More precisely, the antidiuretic hormone of birds, arginine

vasotocin will regulate the allostatic response by adjusting the free water clearance, as well as glomerular filtration rate. In this manner, all this process is orchestrated by kidneys and the lower gastrointestinal tract of birds (cloaca, colon, and digestive ceca), plays an important role in avian osmoregulation (Braun, 2015). On the other hand, wet litter is an important welfare problem in broiler that has not to be neglected in the overall strategies to reduce the use of antibiotics. It is expected that an altered osmoregulation will impact gut permeability, as happens when there is heat stress (Ruff et a., 2020). Under these conditions, it is hypothesized that immune system will be more active because of inflammatory responses induced by endotoxins, mycotoxin and pathogens. The more common nutritionally induced causes of wet litter is polyuria, an increase urine output (Collett et al., 2012).

Regarding Ca and P, decreasing Ca is associated lower litter moisture (Enting et al., 2009; Pos et al., 2003) that led to lower incidence of footpad dermatitis (FPD; Rousseau et al., 2016). However, this effect is more marked when reducing Ca in a low P diets (Rousseau et al., 2012, 2016). Moreover, when comparing 5.5, 7.0 and 8.5 g Ca/kg diet, litter moisture followed a quadratic function with lower value obtained at 7.0 g Ca/kg, and a further reduction when adding 1000 FTU/kg microbial phytase (Herisson et al., 2019). It is worth noting that the diet supplying 7.0 gCa/kg and 1000 FTU/kg phytase resulted in the best growth performance and bone mineralization. Parathormone has been showed to enhance urine volume in birds because it causes an osmotic diuresis due to Ca mobilization from bone and the high P excretion from kidney (Clark and Mok, 1986). Thus, it appears that the underlying mechanism of wet litter is link to an imbalance Ca and P or large excess in both that lead to high plasma concentration, then to osmotic diuresis that then to polyuria, wet litter and even FPD.

3. Meta-analysis as a state-of-the-science tool

Reducing crude protein and balancing amino acids profile: environmental and animal performances lessons
Nitrogen excretion is a major concern in the swine and poultry industry given the implications on environment (e.g. soils acidification, water eutrophication) that has led to worldwide actions aiming to reduce the level of N emissions through different strategies, within them: livestock feeding strategies and low-emission animal housing systems (e.g. Directive EU 2016/2284). To face these challenges, low crude protein (LCP) strategies that are characterized basically by a reduction of soybean meal and a supplementation with limiting AA have reemerged. This strategy works quite well in pigs with a reduction of 2 to 4% CP between NRC (1998) and NRC (2012) (Wang et al., 2018). With the development of industrial synthetic AA technology, supplementary feed grade AA, such as L-valine and L-isoleucine have become available for use in livestock diets, resulting in the potential for further reduction in dietary CP. Nevertheless, a challenge remains in broilers where published researches evaluating the effects of LCP strategies on growth performance of broilers has been conducted through gradient reduction of CP content performed in different ways (ingredients and nutrients) without consensus strategy and with inconsistent results in maintaining growth performance.

A meta-analysis has been performed in broilers (Alfonso-Avila et al., 2019; Minussi et al., 2020) to (1) characterize the nutritional strategies followed in literature in LCP studies and (2) to quantify

the impact of LCP strategies on growth performance, N balance, uric acid, daily water consumption, litter moisture and foot pad dermatitis as well as their main modulating factors. The response variable data were taken from a database of 64 publications including 152 trials in which diets were recalculated based on INRA-AFZ tables (Sauvant et al., 2004). For N balance, only the experiments with constant dietary energy and digestible Lysine content were selected. Since this meta-analysis was performed based on the modulation of the dietary CP, the experiments were independently encoded, thus including a minimum of two dietary treatments (e.g. LCP and control). The studies with more than one growth phases were categorized into two code, 0 to 21 days and 22 to 42 days. Nitrogen balance parameters (intake, excreted, retained and efficiency) were calculated according to Belloir et al. (2017) given only few studies have performed classic N balance. **Recalculations of parameters to generate new Y variables to assess a research question** is another utility of meta-analysis.

Study of the meta-design showed that only 12 experiments have provided essential amino acid at or exceeding 95% of the recommendation (Ajinomoto-Eurolysine S.A.S, 2015; Rostagno, 2011). This confirms that its not possible to study the impact of LCP itself on growth performance by meta-analysis tool, meeting nevertheless one of the objectives of the meta-analysis which is to **take stock of what is available in the literature**. Then, most manuscripts of the database used corn-soybean meal diets. As a result, a decrease in CP was associated with a lower soybean meal, whereas the starch that coming from corn was naturally increased (-1 point % CP = -1.83%; Figure 2a). Also, given the high supply of potassium (K) by soybean meal, its concentration was decreased when decreasing CP (-1 point % CP = 0.05%; Figure 2b) which impacts dietary electrolyte balance (DEB; Figure 2c). Finally, oligosaccharides are also linearly reduced (-1 point % CP = 0.38%; Figure 2d). Therefore, other nutrient than just CP varied in LCP diets.
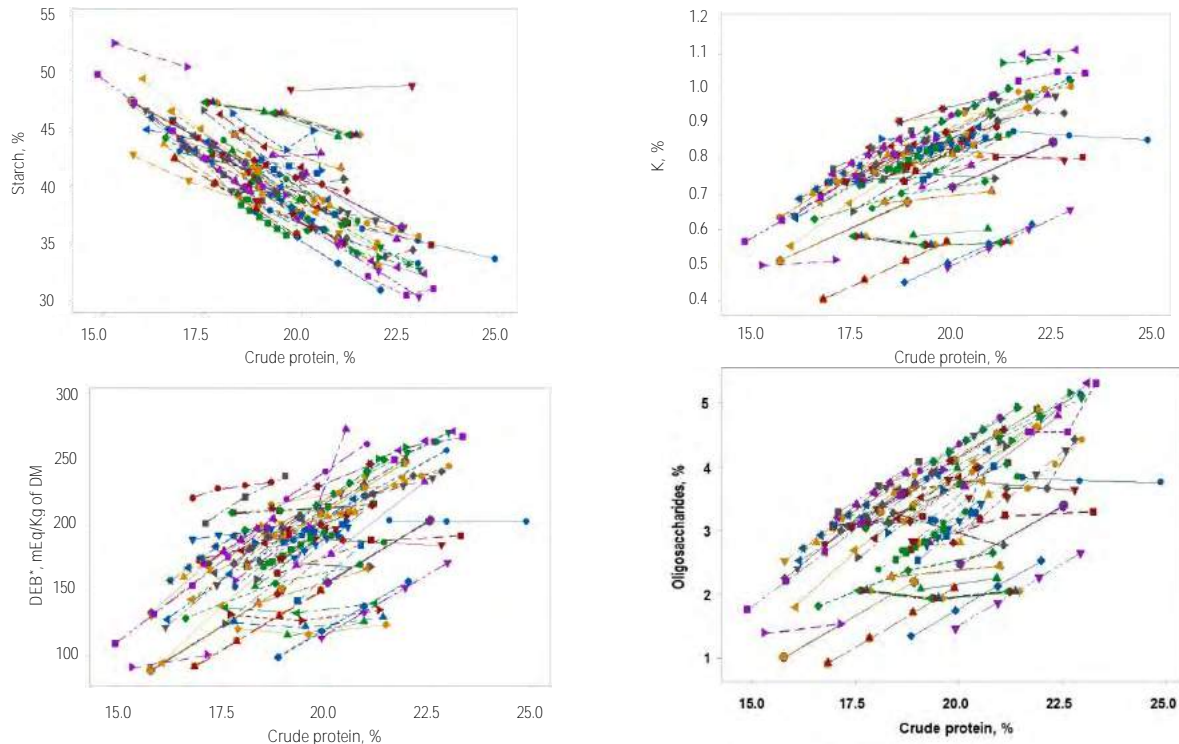
Figure 3. Meta-design: within-experiment relation between (a) starch, (b) Potassium (c) Dietary Electrolyte Balance (DEB) = [Na + K] − [Cl], and (d) Oligosaccharides.

Performed models showed that N efficiency (% intake) was linearly decreases with increasing CP (P<0.001); a decrease 1 % point of CP increases N efficiency by about 2.3%, whereas N excretion was decreased by about 10% (P<0.001). Although not possible to quantify through meta-analysis given the few studies, it is known that this excretion is mainly composed of uric acid (70-80%), that can be converted into ammonia by litter microbes (Ferguson et al., 1998), and of other compounds such as urea, creatinine, and AA (Nahm, 2003; Braun, 2015).

The model generated for litter moisture showed that reducing dietary CP per 1 % point of CP would reduce by 1.2% the litter moisture (P<0.001). The effect on litter moisture seems to be mainly due to the reduction of daily water consumption. The model generated in the current meta-analysis showed a reduction of 7.3 mL/d of daily water consumption per % point CP. Excessive dietary protein supply in birds must be catabolized and excreted via the kidneys in the form of uric acid which implies higher water consumption (McNabb et al.,1973; Chrystal et al., 2019, 2020). Thus, a large urinary flow is required for broilers to excrete an excess of N metabolic products (Nishimura, 2008). In addition, since dietary mineral profile and CP have an additive effect on water consumption (McNabb et al., 1973), reducing the content of soybean meal that supply a significant amount of K (21 g/kg; Figure 2b) and the concomitant change in the DEB (Figure 2c) reduces water consumption, thereby impacting the excreta moisture (Ahmad and Sarwar, 2006).

Litter quality, namely litter moisture and N content, is increasingly important in broilers production, especially because it causes footpad dermatitis (FPD), a tangible welfare issue that can

lead to carcass downgrades. Given the known and now quantified impact of CP on litter moisture, a database including LCP diet and measuring FPD has been built to quantify the impacts of CP on the incidence and severity of this condition (Minussi et al., 2020). The different FPD scoring systems were standardized to footpad score (FPS), varying from 0 to 200. The FPD incidence and FPS linearly decrease (P<0.001) by 15.8% points and 20.5 points (23.2% and 29.1% relatively to the highest CP treatment), respectively, for each % point of dietary CP reduction. In addition, when the FPS of the control treatment is included as a factor in the model, a significant interaction is found between CP and control FPS (P<0.001), indicating that benefit of low protein diets increases with higher initial severity of the condition.

It is worth noting that wet litter being the primary cause of ammonia emissions with N excretion and taken together wet litter and ammonia are one of the most serious performance and environmental factors affecting broiler production (Aziz and Barnes, 2010; Francesch and Brufau, 2003) indicating the LCP strategies in broiler diets is also advantageous in improving health status and animal performance and appears to be a sustainable way of raising broilers in a context of antibiotics growth promoter's removal.

Find alternatives to antibiotic growth promoters
Sustainably improving agricultural productivity to meet increasing demand for animal protein in an ambition to feed 9 billion of people by 2050 is one of the challenges of the 21st century. Sustainability implies affordable, safe, and low footprint production. Regarding safety and especially the increase in antibiotic resistance, the reduction of antibiotics use in livestock production is an urgent public health problem for both human and veterinary medicine and contributes to the dynamic "one Health". In this way, adapted therapeutic strategies are envisaged involving less and better antibiotic use (specific treatment with good dose). To do this, animal and more specifically intestinal health must be monitored with relevant biomarkers using a holistic approach including prevention and early detection of diseases.

The ban of antibiotics use have led to many studies, either to understand the role of antibiotic growth promoters on animals in order to efficiently replace them, or to found alternatives to antibiotic therapeutic but especially to preventive antibiotics to maintain or improve animal health and performance (Gadde et al., 2017). Usually, the sought alternatives are aimed at improving gut health with feed additives such as prebiotics, probiotics, proteobiotics, organic acids, phytobiotics (Vidanarachchi et al., 2005., Gadde et al., 2017). However, there is no clear definition of gut health. It refers to positive aspects of the gastrointestinal (GI) tract, such as effective digestion of feed and absorption of nutrient, the absence of GI illness, normal and stable intestinal microbiota, and effective immune status. Even more complicated is how gut health can be measured? All these considerations have fueled the search for biomarkers of the digestive inflammation in swine and poultry, but there is still no gold standard and the solution will probably involve a panel (Ducatelle et al., 2018).

Several reviews have tried to highlight the effects of alternatives to antibiotic growth promoters (AGP), but with little success given the wide responses variation. To provide an overview of the alternatives that have been evaluated *in vivo* and quantify their impacts in terms of growth performance in comparison to negative controls was performed in broilers (Rouissi et al., 2020), as previously done in pig (Schweer et al., 2017). The objective of this study was (1) to identify the

most selected alternatives to AGP for broilers and to quantify their effects on growth performance relative to an antibiotic-free control diet and (2) to shed light on the effective modulating factors.

The information was extracted from peer-reviewed papers published from 2000 to 2017 and retrieved from various public databases. This selection of databases contains articles of enough diversity to make sampling bias unlikely. The keywords used in the search were: *'chicks' or 'poultry' or 'broiler'* + *'prebiotic', 'essential oil', 'probiotic', 'organic acid' and 'performance', 'alternatives', 'necrotic enteritis'*. This results in 130 publications. Then the articles were retained for the meta-analysis if they met the following criteria: (1) broiler trial fed with non-antibiotic growth promoter, (2) inclusion of an antibiotic-free control diet (C-), (3) reporting of feed conversion ratio, (4) diet composition, and (5) broiler genetic line. Only 79 publications totaling 150 experiments were kept. They were grouped into four sub-database categories, namely organic acids (Nexp = 30), prebiotics (Nexp = 50), probiotics (Nexp = 30), and essential oils (Nexp = 40). In each of the databases a product largely dominated, i.e. butyrate (70%) for organic acids, mannan-oligosaccharides (MOS; 84%) for prebiotics, *Bacillus subtilis* (77%) for probiotics, and origan based essential oils (65%) and they were thus isolated in 4 other sub-database and studied. Given large difference in doses and broilers age it was not possible to compare statistically these four products, they were thus studied independently. It should be noted that there is no doubt that this meta-analysis is subject to publication bias, negative or inconclusive trials being often non-published.

Quantifying the effects of alternative to antibiotics growth promoters on growth performances.
Average daily weight gain (ADG), average daily feed intake and feed conversion ratio (FCR) were modeled as Y variables. No effect on feed intake was found, whereas daily gain increased linearly and quadratically (P<0.05) with dose of butyric acid, mannan-oligosaccharides and essential oil and linearly with *Bacillus subtilis* by up to respectively 7%, 8%, 7% and 9%. Metabolizable energy content had a linear effect (P < 0.05) on feed conversion ratio in butyric acid and MOS databases. Improvement in FCR reached 2.4%, 8%, 7% and 3% respectively for butyric acid, MOS, essential oil and *Bacillus subtilis*. By quantifying the effect of non-antibiotic growth promoters, the model proposes a rational basis for their use in broiler diets. These alternatives have then been tested in the field (Rouissi et al., 2019) but some of then did not resulted in the same effect on growth performance showing that studies around their mode of action to finetune their use are still needed.

Highlighting modulating factors of alternative to antibiotics growth promoter's response.
Given inconsistencies in the results found in the literature on alternatives to AGP, with equivalent or even better responses than antibiotics while other reported no effect, a specific focus on founding modulating factor of their response have been also done. This has been tested in two works, the one of Rouissi et al. (2018, 2020) just described and the one of Létourneau-Montminy et al. (2019) and Gabarrou et al. (2020) that have evaluated the effects of a specific blend of oleoresins of spices and essential oils (Oleo) based on an exhaustive database including 25 trials with Oleo allowing to quantify its impact on ADG and FCR of broilers.

In both meta-analysis, feed composition was used to recalculate the dietary nutrient profile, especially apparent metabolizable energy (AME), crude protein and digestible amino acids, from INRA tables of feedstuffs (Sauvant et al., 2004). The AME requirement, standardized ileal

digestibility of lysine (SID Lys) and CP values were then compiled based on bird genetic line (ROSS 308, 2014; COBB 500, 2015) and the dietary concentration in each experiment was expressed in % of the requirement. In addition to the continuous dose of non-antibiotic alternative, the dietary provision relative to requirement, the genetic line, the presence of challenge (pathogenic bacteria administered orally) and the bird age (starter, grower, finisher) were tested, as well as their interactions. Also, in the works of Létourneau-Montminy et al. (2019) and Gabarrou et al. (2020) another strategy was implemented with the objective of testing the hypothesis that alternative to AGP response was influenced by the challenge the bird experienced. Therefore, when a positive control (C+) with antibiotic growth promoter and a negative control (C-) without antibiotic were both present in the same trial (n=9), the relative difference between them (C+/C-) was also calculated as *Challenge Acuity Index* and used as an X variable.

An interaction between AME in % of requirement and prebiotic effect have been found by Rouissi et al. (2018) showing that the more the bird is energy deficient the more its response to prebiotics was important. Besides, Létourneau-Montminy et al. (2019) and Gabarrou et al. (2020) showed that the effect of Oleo of FCR expressed in percentage of C- was negatively correlated (Linear, P=0.03; Quadratic, P=0.02; R2=85%) with the Challenge Acuity Index (C+/C-) indicating that in challenge condition (when C+ performed better than C-), the reduction of FCR with Oleo addition was higher (Figure 4). This new index is interesting to better understand the variations observed between studies for a similar alternative to AGP and will be for sure reuse in the future.
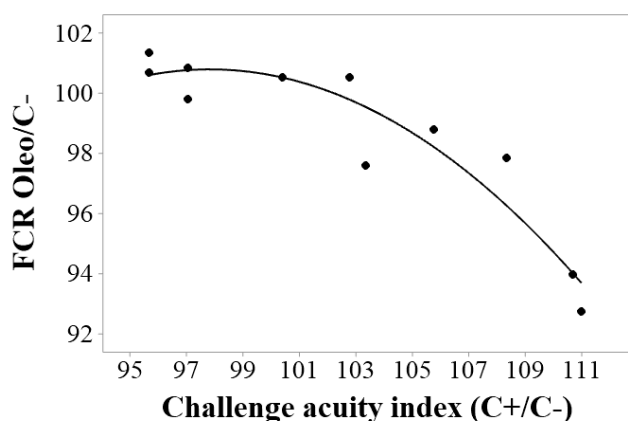
Figure 4. Relationship between the effect of feed conversion ratio (FCR) of bird receiving Oleo in comparison to negative control (C-) without antibiotic and the difference in terms of FCR between control positive with antibiotic (C+) and C-.

Conclusion

The matching of the constant demand for animal proteins and the genetic progress we have witnessing in the last decades is a challenge, especially from nutrition point of view. In parallel, there is an unprecedented increase in data available on a subject. For these advances to translate into affordable and safe animal protein produce with a minimal environmental footprint, adequate

and precise nutritional management is necessary. This undoubtedly involves multi-criteria methods adaptable to different production contexts.

By incorporating the results of numerous publications into meta-analysis approaches, insights to improve broiler and pig nutrition and health are proposed. Indeed, there is an equilibrium to find between Ca and P to fulfil tissues growth without too much excesses to maintain a good litter quality and animal health. This strategy, that deserves more attention in pig, can even be a lever to reduce the use of antibiotics. Although meta-analysis and modeling approaches are ongoing and will help to make progress, animal trials on microbial phytase of second generation are still needed to fine-tune the equilibrium given few data available in our database and the fact that they are a source of P. Besides, in broilers, meta-analysis clearly showed that reducing CP in broilers is a way of improving health by improving litter quality and reducing ammonia emissions this with a lower environmental footprint, health effects expected to be similar in pigs. In broilers, the maintenance of growth performance is however challenging but is the subject of many recent studies and it can therefore be expected that effective strategies for protein decreases will be developed. Finally, some alternatives to AGP has been found and their effects quantify in terms of growth performance. However, other biomarkers must be studied and proposed to better understand the variation in the effects found in the field.

Meta-analysis is now widely accepted and applied in animal sciences, especially in nutrition. Its implications in systemic approaches and mechanistic modeling is promising. Precision farming and large datasets facing heterogeneity and lack of data to explain it are in the crosshairs of meta-analysis. There is also a growing interest in using meta-analysis to interpret individual laboratory databases by grouping the results of various experiments.

References

Ahmad, T. and M. Sarwar. 2006. Dietary electrolyte balance: Implications in heat stressed broiler. World's Poult. Sci. J., 62: 638-653.

Ajinomoto Eurolysine SAS. 2015. Go to essentials; Amino acid solutions for lower crude protein levels in broilers feeds. Information 38.

Alfonso-Avila, R.A., O. Cirot, W. Lambert and M.P. Létourneau-Montminy. 2020. Effect of low protein diets on nitrogen utilization and litter quality of broilers insights from a meta-analysis. Anim. Submitted

Applegate, T.J., R. Angel and H.L. Classen. 2003. Effect of dietary calcium, 25-hydroxycholecalciferol, or bird strain on small intestinal phytase activity in broiler chickens. Poult. Sci. 82 1140-1148.

Aziz, T., H.J. Barnes. 2009. Harmful effect of ammonia on birds. World Poult 26 28-30

Bell, A. W. 1995. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. J. Anim. Sci. 73 2804-2819.

Bauman, D.E., L.H. Baumgard, B.A. Corl and J.M. Griinari. 2000. Biosynthesis of conjugated linoleic acid in ruminants. Proc. Am. Soc. Anim. Sci., 1999. Available at: http://www.asas.org/jas/symposia/ proceedings/0937.pdf

Belloir, P., B. Meda, W. Lambert, E. Corrent, H. Juin, M. Lessire and S. Tesseraud. 2017. Reducing the CP content in broiler feeds: impact on animal performance, meat quality and nitrogen utilization. Anim. 11 1881-1889.

Braun E. 2015. Osmoregulatory-systems-of-birds In Osmoregulatory Systems of Birds 2015 Eldon J.Braun Sturkie's Avian Physiology: Sixth Edition 285-300 Elsevier Inc.

Chrystal, P.V., A.F. Moss, A. Khoddami, V.D. Naranjo, P.H. Selle and S.Y. Liu. 2020. Impacts of reduced-crude protein diets on key parameters in male broiler chickens offered maize-based diets. Poult. Sci. 99 505-516.

Chrystal, P.V., A.F. Moss, A. Khoddami, V.D. Naranjo, P.H. Selle and S.Y. Liu. 2019. Effects of reduced crude protein levels, dietary electrolyte balance, and energy density on the performance of broiler chickens offered maize-based diets with evaluations of starch, protein, and amino acid metabolism. Poult. Sci. 99 1421-1431.

Clark, N.B. and L.L. Mok. 1986. Renal excretion in gull chicks: effect of parathyroid hormone and calcium loading. Am. J. Physiol. 250, R41–R50

Collett. S.R. 2012. Nutrition and wet litter problems in poultry. Anim. Feed Sci. Techn. 173 65-75.

COBB 500 Performances et Recommandations Nutritionnelles. 2015. Accessed Feb. 2017. https://cobbstorage.blob.core.windows.net/guides/27811ef0-5d15-11e8-9602-256ac3ce03b1.

Couture, C., B. Meda, A. Narcy, M.P. Létourneau-Montminy. 2019a. Méta-analyse de la digestibilité iléale du phosphore chez le poulet de chair : effet du phosphore alimentaire, du calcium et de la phytase, Journées de la recherche avicole et palmipèdes à foie gras, 13 155-156.

Couture, C., B. Meda, A. Narcy, M.P. Létourneau-Montminy. 2019b. Modélisation de l'utilisation du phosphore alimentaire chez le poulet de chair, Journées de la recherche avicole et palmipèdes à foie gras, 13 90-95.

Crenshaw, T.D. 2001. Calcium, phosphorus, vitamin D, and vitamin K in swine nutrition. In Swine nutrition (ed. AJ Lewis and LL Southern), 2nd edition, pp. 187–212. CRC Press, Boca Raton, FL.

De Rauglaudre, T. and M.P. Létourneau Montminy. 2020. Prediction of urinary phosphorus excretion as a tool to assess mineral status of growing pigs. J Anim Sci. 98 (Suppl. 2) 18.

Enting, H., J. del los Mozos, A. Gutiérrez del Álamo, P. Pérez de Ayala. 2009. Influence of minerals on litter moisture. 17th Eur. Symp. Poult. Nutr.WPSA, Edinburgh, UK, pp. 47–52

FAO. http://www.fao.org/animal-production/en/?%DC%FB%BF%07

Ferguson, N.S., R.S. Gates, J.L. Taraba, A.H. Cantor, A.J. Pescatore, M.L. Straw, M.J. Ford and D.J. Burham. 1998. The Effect of Dietary Protein and Phosphorus on Ammonia Concentration and Litter Composition in Broilers. Poult. Sci. 77 1085-1093.

Flachowsky, G., and J. Kamphues. 2012. Carbon Footprints for Food of Animal Origin: What are the Most Preferable Criteria to Measure Animal Yields? Animal 2 108-126.

Francesch, M. and J. Brufau. 2004. Nutritional factors affecting excreta/litter moisture and quality World's Poult. Sci. J. 60 64-75.

Gabarrou, J.F., F. Messad, B. Medina, M.P. Létourneau-Montminy. 2020. Effects of a specific blend of oleoresins of spices and essential oils on growth performance of broilers is influence by challenge acuity. IPSF, Atlanta January 28-30th

Gadde, U., Kim, W.H., Oh, S.T., Lillehoj, H. 2017. Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry: a review. Animal Health Research Reviews. 18. 1-20.

Heaney, R.P. and B.E.C. Nordin. 2002. Calcium effects on phosphorus absorption: implications for the prevention and co-therapy of osteoporosis. J. Am. Coll. Nutr. 21 239–244.

Hedhli, A., M. Hamdi, A. Narcy and M.P. Létourneau-Montminy. 2019. Meta-analysis of the impact of dietary phosphorus, calcium and microbial phytase on growth performance in broilers. European Symposium of Poultry Nutrition, Gdansk Pologne, June 10-14th.

Herisson, C., C. Couture and A. Narcy, M.P. Létourneau-Montminy. 2019. Exploring the capacity of the chicken to catch-up in growth and bone mineral deficit depending on P and Ca levels. PSA Annual Meeting, P. Sci Vol. 98, E-Suppl. 1, 566

Kornegay, E.T. and H. Qian. 1996. Replacement of inorganic phosphorus by microbial phytase for young pigs fed on a maize–soyabean-meal diet. British Journal of Nutrition 76 563–578.

Létourneau-Montminy, M.P., F. Messad, B Medina and J.F. Gabarrou. 2019. Effects of a specific blend of oleoresins of spices and essential oils on growth performance of broilers: Insight from a meta-analysis approach. Poult. Sci Vol. 98, E-Suppl. 1, 659

Létourneau-Montminy, M.P., A. Narcy, T.D. Crenshaw, J.Y. Dourmad and C. Pomar. 2015. Modeling the metabolic fate of dietary phosphorus and calcium in growing pigs and the dynamics of body ash. J. Anim. Sci. 93 1200-1217

Létourneau-Montminy, M.P. and A. Narcy. 2013. Meta-analysis of the impact of dietary phosphorus, calcium and microbial phytase on growth performance in pigs. J Anim Sci. 91 (Suppl. 2) :100.

Létourneau-Montminy, M.P., C. Jondreville, D. Sauvant and A. Narcy. 2012. Meta-analysis of phosphorus utilisation by pigs: influence of dietary calcium and microbial phytase, Anim. 6 1590-1600.

Mcnabb. R., F. Menabb, A. Hinton, 1973. The Excretion of Urate and Cationic Electrolytes by the Kidney of the Male Domestic Fowl (Gallus domesticus). J. comp. Physiol 82 pp: 47-57

Minussi, I., W. Lambert and M.P. Létourneau-Montminy. 2020. Quantification of the benefits of low protein diets on foot pad dermatitis incidence and severity in broiler chickens. Poultry Science Meeting Abstract, Submitted

Nahm, K.H. 2003. Evaluation of the nitrogen content in poultry manure. World's Poult. Sci. J.. 59 77-88

Narcy, A., Létourneau-Montminy M.P., Dourmad J.Y. 2012. Modulation de l'utilisation digestive du phosphore chez le porcelet sevré: influence de l'apport de calcium et de phytase sur le pH et la solubilité des minéraux au niveau gastro-intestinal J. Rech. Porc. 44 159-164.

Nishimura, H. 2008. Urine concentration and avian aquaporin water channels Pflugers Archiv European Journal of Physiology 456 (4) pp: 755-768.

NRC. 1998. Nutrient requirements of swine. 10th ed. Natl. Acad. Press, Washington, DC

NRC. 2012. Nutrient requirements of swine. 11th ed. Natl. Acad. Press, Washington, DC

Pos, J., Enting, H., Veldman, A., 2003. Effect of phytase and dietary calcium level on litter quality and broiler performance. Proc. 14th Eur. Symp. Poult. Nutr. 17–18. World's Poultry Science Association, Lillehammer, Norway.

Qian, H., Kornegay, E.T. and D.E. Conner Jr. 1996. Adverse effects of wide calcium: phosphorus ratios on supplemental phytase efficacy for weanling pigs fed two dietary phosphorus levels. Journal of Animal Science 74 1288–1297.

Rostagno, H.S., L.F. Teixeira Albino , J.L. Donzele, P.C. Gomes, R.F. de Oliveira, D.C. Lopes, A.S. Ferreira, S.L. de Toledo Barreto and R.F. Euclides. 2011. Tabelas Brasileiras para aves e suinos: Composição de Alimentos e Exigências Nutricionais, 3ªEdição. Universidade Federal de Viçosa–Departamento de Zootecnia, Viçosa, MinasGerais, Brazil, 252pp. Sauvant D, Perez JM

ROSS 308 BROILER: Nutrition Specifications. 2014. Accessed Feb. 2017. http://tmea.staging.aviagen.com/assets/Tech_Center/Ross_Broiler/Ross-308-Broiler-Nutrition-Specs-2014r17-EN.pdf.

Rouissi A, Guay F, Létourneau Montminy MP. 2018. Towards the replacement of antibiotics growth promoters in chicken: meta-analysis approach. Proceeding. Gut health symposium, st-louis, États-Unis, Abst. 125

Rouissi, A., F. Guay, M. Boulianne and M. P. Létourneau-Montminy. 2019a. Effects of butyrate, mannan-oligosaccharide, Bacillus subtillis and ß-glucans from naked oat on growth performance of broilers. Poult. Sci Vol. 98, E-Suppl. 1, 659

Rouissi, A., F. Guay, M. Boulianne and M. P. Létourneau-Montminy. 2020. Assessing growth promoter effect and modulating factors of dietary feed additive commonly used in broilers: insights from meta-analysis approach. Poult. Sci., Submitted.

Rousseau, X., M.P. Létourneau-Montminy, N. Même, Magnin M., Y. Nys and A. Narcy. 2012. Phosphorus utilization in finishing broiler chickens: effects of dietary calcium and microbial phytase. Poult. Sci. 91 2829-2837.

Rousseau, X., A.S. Valable, M.P. Létourneau-Montminy, N. Même, E. Godet, M. Magnin, Y. Nys, M. Duclos and A.Narcy. 2016. Adaptive response of broilers to dietary phosphorus and calcium imbalance. Poult. Sci. 95 2849:2860.

Ruff, J., T.L. Barros, G. Tellez Jr., J. Blankenship, H.Lester, B.D. Graham, C.A.M. Selby, Christine N. Vuong, S. Dridi, E.S. Greene, X. Hernandez-Velasco, B.M. Hargis and G. Tellez-Isaias. 2020. Research Note: Evaluation of a heat stress model to induce gastrointestinal leakage in broiler chickens. Poult. Sci. 99 1687–1692.

Sauvant, D., J.M. Perez and G. Tran. 2004. Tables of composition and nutritional value of feed materials. Institut National de la Recherche Agronomique, Association Franc¸aise de Zootechnie, Paris, France.

Sauvant, D., P. Schmidely and J.J. Daudin. 2005. Méta-analyse des données expérimentales: applications en nutrition animale. INRA Prod. Anim., 18 63-73.

Sauvant, D., P. Schmidely, J.J. Daudin and N.R. St-Pierre. 2008. Meta-analyses of experimental data in animal nutrition. Animal. 2 1203–1214.

Sauvant, D., M.P. Létourneau-Montminy, J.B. Daniel JB, P. Schmidely, M. Boval and C. Loncke. 2019. Use and misuse of meta-analysis in animal science, (Papers presented at the 9th International Workshop on Modelling Nutrient Digestion and Utilisation in Farm Animals) Animal (Full text), accepted.

Saint-Pierre, N. 2001. Invited Review: Integrating Quantitative Findingsfrom Multiple Studies Using Mixed Model Methodology. J. Dairy Sci. 84 741-755.

Schweer, W. P. J. F. Patience, K. Schwartz, D. Linhares, C. Rademacher, H. K. Allen, C. L. Loving, A. Ramirez, and N. K. Gabler.2017. A review and evaluation of antibiotic alternatives in the literature. *Journal of Animal Science*, 95, Suppl_2 148.

Sebastian, S., S.P. Touchburn, E.R. Chavez, and P.C. Lague. 1996. Efficacy of supplemental microbial phytase at different dietary calcium levels on growth performance and mineral utilization. Poult. Sci. 75 1516–1523.

Suttle N.F. 2010. Mineral nutrition of livestock, 4th edition. CABI Publishing, Wallingford, Oxfordshire, UK.

Valable A.S. 2018. Exploring the adaptive potential of broilers to optimize the use of phosphorus and dietary calcium. PhD Thesis, Laval University, Canada -Francois Rabelais, France.

Vidanarachchi, J., H. Mikkelsen, I. Sims, P. Iji, M. Choct. 2005. Phytobiotics: Alternatives to antibiotic growth promoters in monogastric animal feed. Recent Advances in Animal Nutrition in Australia. Vol. 15. 131-144.

Wang, Y., J. Zhou, G. Wang, S. Cai, X. Zeng and S. Qiao. 2018. Advances in low-protein diets for swine. J. Anim. Sci. Biotech. 9 60-74.

Feed Processing for Optimal Animal Health and Performance

Transformation des aliments et optimisation de la santé et du rendement des animaux

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Abstract

Feed processing methods impacts the health and performance of animals. Grain cleaning, seed sorting to remove toxic seeds or materials, particle size reduction, conditioning, pelleting and extrusion health can all affect the health status of the animal including gut health. The impacts of these processes are not consistent between species of livestock and can require customization within species. For example, the impact of fine particles of starch resulting from grinding can have negative consequences on beef and dairy rumen health however, these effects can be acute in nature and therefore are actively managed. On the other hand, fine grinding increases digestibility in swine but too fine of grind can cause stomach ulcers which are not easily observed and the impact is more chronic and therefore not as actively managed. Traditionally grains are finely ground to increase pellet quality and digestion rate by poultry. This reduces retention time in the gizzard and proventriculus therefore reducing exposure of microorganisms to gastric acid which would otherwise prevent viable pathogens from entering the small intestine. Hydrothermal treatments such as conditioning, extrusion or steam explosion can reduce pathogen loads in feed but will alter the rate and extent of digestion of some nutrients such as starch, fibre encapsulated nutrients or protein and this too can affect gut health. The feed industry relies on cost effective feed ingredients but some are low quality materials. In some cases it may make sense to preclean or sort the lower quality grains to reduce the potential negative effects of mycotoxins such as deoxynivalenol, ergot alkaloids or plant based toxins found in weed seeds. In light of restrictions on the use of antibiotics in feed in December of 2018 in Canada, effective preventative strategies

to manage enteric disease are more important than ever. The objective of this presentation is to discuss the how each of these feed processes may affect the health and performance of animals.

Résumé

Les méthodes de transformation des aliments ont une incidence sur la santé et le rendement des animaux. Le nettoyage des grains, le tri des semences pour éliminer les graines ou les matières toxiques, la réduction de la taille des particules, le conditionnement, l'agglomération et l'extrusion sont autant d'éléments qui peuvent avoir une incidence sur l'état de santé de l'animal, y compris sa santé intestinale. Les répercussions de ces procédés ne sont pas uniformes d'une espèce animale à l'autre et peuvent nécessiter certaines adaptations au sein même de chaque espèce. Par exemple, l'impact des fines particules d'amidon résultant du broyage peut avoir des conséquences négatives sur la santé du rumen des bovins de boucherie et laitiers et on y porte une grande attention car les effets peuvent être aigus. D'autre part, le broyage fin augmente la digestibilité chez les porcs, mais une mouture trop fine peut provoquer des ulcères d'estomac qui ne sont pas facilement observables; les conséquences étant de nature plus chronique, la gestion de cette pratique alimentaire est moins serrée. Traditionnellement, les grains sont broyés finement pour augmenter la qualité des granulés et le taux de digestion chez les volailles. Cela réduit le temps de rétention dans le gésier et le proventricule, réduisant ainsi l'exposition des microorganismes à l'acide gastrique, un mécanisme qui, normalement, empêche des agents pathogènes viables de pénétrer dans le petit intestin. Les traitements hydrothermiques tels que le conditionnement, l'extrusion ou la fragmentation par la vapeur, peuvent réduire la charge en agents pathogènes des aliments, mais ils modifient la vitesse et le taux de digestion de certains nutriments tels que l'amidon, les éléments encapsulés dans des fibres ou les protéines, ce qui peut aussi nuire à la santé intestinale. L'industrie de l'alimentation animale repose sur l'utilisation d'ingrédients alimentaires économiques, dont certains sont toutefois de qualité inférieure. Il est parfois judicieux d'effectuer un premier nettoyage de l'ingrédient ou de trier les grains de moindre qualité afin de réduire les effets négatifs potentiels des mycotoxines telles que le désoxynivalénol, les alcaloïdes de l'ergot ou les toxines végétales présentes dans les graines de mauvaises herbes. Compte tenu des restrictions imposées sur l'utilisation d'antibiotiques dans les aliments pour animaux depuis décembre 2018 au Canada, il est plus important que jamais d'adopter des stratégies efficaces pour prévenir les maladies entériques. L'objectif de cet exposé est de discuter de la manière dont chacun de ces procédés de transformation des aliments peut influer sur la santé et le rendement des animaux.

The role of livestock in sustainable food production systems in Canada

Le role du bétail dans les systèmes de production durables au Canada

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Abstract

Global drivers such as the growing human population, continuously evolving consumer preferences, globalization, and climate change have put pressure on the agri-food sector to produce more livestock products with less land, less feed and less water. Taste, nutritional value, cost, source and environmental sustainability of food has become an essential element of purchasing decisions for many consumers. Indeed, an environmental footprint composed of greenhouse gas emissions, nutrient and water use efficiency, water quality, carbon storage and biodiversity has been completed for many commodities. However, as livestock production systems are complex agro-ecosystems that typically include each of these parameters, it is extremely challenging to establish a single value to assess overall sustainability. This complexity presents a grand challenge to all stakeholders in the livestock value chain – producers, processors, retailers, nutritionists and researchers - to improve the sustainability of our agro-ecosystems, as well as to share this information with the consumer. There is no silver bullet to solve the environmental concerns of all livestock production systems as they operate under different constraints on different landscapes, with different water and nutrient cycles, plant and soil types and a myriad of other factors. Further, the lack of scientific evidence regarding the interactions between livestock production, human nutritional adequacy, and the health of our environment makes it difficult for consumers to interpret this information and make informed food choices. This presentation will examine these complex interactions and trade offs, as well as the potential impacts of changes in consumer dietary

choice on environmental sustainability, nutritional adequacy and land use. It will also challenge the agricultural sector to consider strategies by which we can engage with consumers in more fulsome conversations regarding the sustainability of livestock production systems.

Résumé

Certains facteurs de portée mondiale, tels que la croissance de la population humaine, l'évolution constante des préférences des consommateurs, la mondialisation et le changement climatique, ont fait pression sur le secteur agroalimentaire pour qu'il accélère la production animale avec moins de terres, moins d'aliments et moins d'eau. La saveur, la valeur nutritionnelle, le coût, la source et la viabilité écologique des produits alimentaires sont devenus un élément essentiel des décisions d'achat pour de nombreux consommateurs. En effet, l'empreinte environnementale, comprenant les émissions de gaz à effet de serre, l'efficacité de l'utilisation des nutriments et de l'eau, la qualité de l'eau, le stockage du carbone et/ou la biodiversité, a été calculée pour de nombreux produits de base. Toutefois, comme les systèmes de production animale sont des agro-écosystèmes complexes généralement soumis à chacun de ces paramètres, il est extrêmement difficile d'établir une valeur unique pour décrire leur caractère durable à l'échelle planétaire. Cette complexité représente un grand défi pour tous les acteurs de la chaîne de valeur de l'industrie animale - producteurs, transformateurs, détaillants, nutritionnistes pour animaux et chercheurs - soucieux d'améliorer la durabilité de nos agro-écosystèmes ainsi que de partager ces informations avec le grand public. Il n'existe pas de solution miracle pour résoudre les problèmes environnementaux associés à tous les systèmes de production animale, car ils fonctionnent sous différentes contraintes, dans différents milieux, avec différents cycles de l'eau, différents types de plantes et de sols et une foule d'autres facteurs. En outre, le manque de preuves scientifiques concernant les interactions entre la production animale, la suffisance nutritionnelle et la qualité de l'environnement fait qu'il est difficile pour les consommateurs d'interpréter ces informations et de faire des choix alimentaires éclairés. Cet exposé porte sur ces interactions complexes, ainsi que sur les impacts potentiels des nouveaux choix alimentaires des consommateurs sur la durabilité environnementale, la suffisance nutritionnelle et l'utilisation des terres. Cela mettra également le secteur agricole au défi d'envisager des stratégies permettant d'engager avec les consommateurs des conversations plus approfondies sur la durabilité des systèmes de production animale.

Global drivers impacting food production and consumption

The agricultural sector is faced with the daunting challenge of producing food for a growing global population, which is expected to reach 8.5 billion by 2030, 9.7 billion by 2050 and 11.2 billion by 2100 (UN WPP 2019), with sub-Saharan African countries accounting for more than half of the growth of the world's population between 2019 and 2050 (Figure 1; UN WPP 2019).
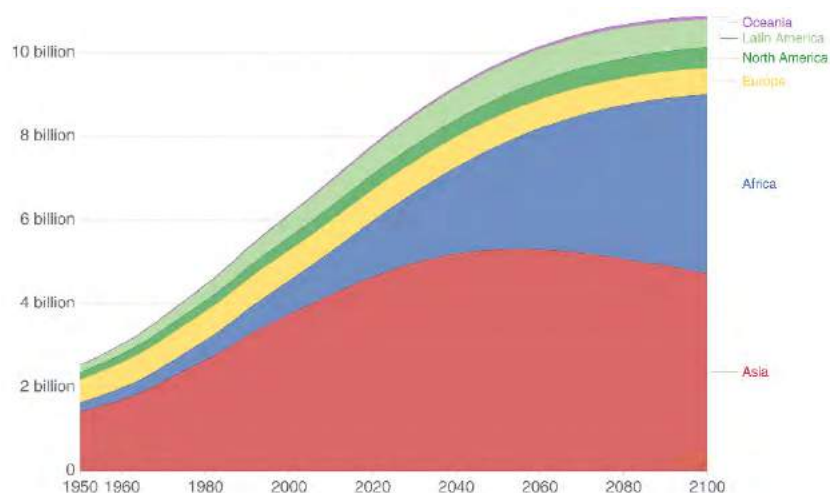
Figure 1: World population by region projected to 2100, based on the United Nation's medium population scenario (UN WPP 2019).

Population growth, along with increased socio-economic status and urbanization (Motett et al., 2017) are expected to increase global demand for meat and milk by 57% and 47%, respectively (Alexandratos and Bruinsma, 2012). The majority of growth is expected to occur in developing countries (Motett et al., 2017). The East and Southeast Asian region is expected to realize income grow of 60-100% /capita by 2028, resulting in a greater demand for meat, leading to a 5 kg/capita increase in China and 4 kg/capita increase in Southeast Asia, largely due to greater poultry and pork consumption, the two meats most widely consumed in those regions. Beef consumption in China is also expected to rise by 0.5 kg/capita over the next decade, bringing average consumption to 4 kg/capita. In South Asia, income growth is projected to be associated with greater consumption of dairy products, sugar and vegetable oil. Dairy products and pulses will remain critical sources of protein within this region. Pakistan is expected to lead global dairy consumption growth, with annual consumption of 274 kg/capita, nearly 30% of total daily per capita protein availability. Dairy consumption is projected to grow in India as well, and will account for 15% of total per capita protein intake by 2028 (OECD-FAO, 2019). This growth in the global demand for animal-based protein could present opportunities for growth in Canadian export markets.

Over the last decade, consumption of chicken has increased, while consumption of beef, pork and fluid milk has decreased in Canada. However, a recent Canadian survey (n=1029) revealed that more than 48% of respondents stated that they consume meat daily while 40% consume meat once or twice per week (Charlebois et al., 2020). Further, 82% stated that they do not have dietary restrictions, 10% considered themselves flexitarians, 1.2% were pescetarian, 1.1% were vegan, 1.2% were lacto-ovo vegetarian and 2.1% were vegetarian (Charlebois et al., 2020).

Consumer preferences and consumption of animal-based foodstuffs

Cultural appropriateness, taste, nutritional value, cost, source, availability, ethical considerations and environmental sustainability of food products influence the purchasing decisions and

consumption patterns for many consumers. The nutritional attributes of livestock commodities are well documented. Red meat is a source of several essential trace elements, often present in a highly-absorbable form (Rooke et al., 2010; Williamson et al., 2005). Animal-based food products provide a significant portion of the essential fatty acids (23-100%) and amino acids (34-67%) available for human consumption in the U.S. (White and Hall, 2017).

In the last decade, links between diet, nutrition and environmental sustainability have increased the complexity of diet selection for consumers as they search for a sustainable diet. The FAO defines sustainable diets as those with low environmental impacts which contribute to food and nutritional security and to a healthy life for present and future generations. Sustainable diets are protective and respectful of diversity and ecosystems, cultural preferences, accessible, economically affordable, nutritionally adequate, safe and healthy, while optimizing natural and human resources (FAO, 2010). The sustainability of animal-based diets has been widely criticized, with an emphasis on the adoption of plant-based diets to reduce global agricultural greenhouse gas emissions, reduce land clearing and improve human health outcomes (Tilman and Clarke, 2014; Willett et al., 2019). A simulation study examining the GHG impacts of removing animals from the US agricultural production system projected a meniscal overall reduction in GHG emissions of 2.6% (White and Hall, 2017).

Consumer preferences regarding best practices associated with animal-based products can also impact environmental sustainability. Studies in the US (Capper and Hayes, 2012) and Canada (Basarab et al., 2012) have shown that use of productivity enhancing technologies such as implants, ionophores and beta agonists led to a 5 to 10% reduction in GHG and ammonia emissions, as well as a 10% reduction in land use required to produce the same quantity of beef.

Environmental footprint of animal-based products in Canada

Consumer interest in environmental sustainability has led to an examination of the carbon footprint of both animal- and plant-based agricultural commodities. An environmental footprint of a product is a valuation of sustainability indicators including GHG's, nutrient, land and water use efficiency, water quality, carbon storage and biodiversity throughout the supply chain. The choice of the functional unit, which for GHG's can be expressed as net emissions, emissions per unit of commodity expressed on a weight basis, or emissions per kg of nutrient can significantly influence study outcomes. For example, GHG emissions of processed fruits and vegetables expressed on a weight basis were lower than meat and meat products, milk and dairy products, grain and other foods, as well as sweets (Drewnowski et al., 2015). However, when expressed per 100 kcal of energy, vegetables had the highest emissions relative to all other categories. Several studies have also examined the relationship between the nutrient density of foods and GHG emissions, demonstrating that animal-based commodities have consistently lower emissions when adjusted for energy (Vieux et al., 2013), protein (Veeramani et al., 2017) or overall nutrient density (Brunn Werner et al., 2014) compared to if the emissions for that commodity were expressed on a weight basis.

A novel approach to assess the sustainability of livestock production systems is a comparison of global feed conversion ratios (protein MT/year, kg dm/kg protein, kg edible dm/kg protein, kg edible dm/kg meat, kg complete dm/kg protein, kg edible protein/kg protein) as described by Mottet et al., (2017). These researchers report that of the 6 billion tonnes of feed consumed annually (including 1/3 of annual global cereal production), 86% of which is considered unsuitable for consumption by humans. Furthermore, Mottet et al. (2017) estimated that on a global basis, an average of 2.8 and 3.2 kg of potentially human-edible feed are required to produce 1 kg of boneless meat in ruminant and non-ruminant production systems, respectively; values well below that which are often cited in the literature.

In Canada, agriculture accounts for 8.1% of total greenhouse gas emissions (Figure 2, Environment Canada 2019) and although this is a small contribution compared to other sectors, environmental footprints have been established for several commodities including beef, dairy and eggs.
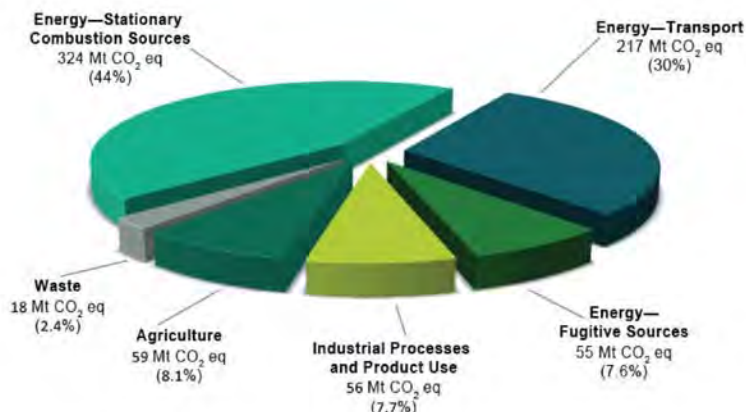
Figure 2. Breakdown of Canada's greenhouse gas emissions by sector in 2018.

Over a 30-year time period (1981-2011), Canadian beef producers have reduced GHG emission intensity by 15% (Legesse et al., 2016) with 24% less land, ammonia emission intensity by 17% (Legesse et al., 2018) and water use intensity by 20% (Legesse et al., 2018). Similarly, in another study conducted in the US, the nation's beef industry in 2007 required 70% of the beef cattle, 88% of the water, and 67% of the land required in 1977, to produce the same amount of beef while the carbon footprint per unit of beef declined by 16% (Capper, 2011). An environmental footprint has also been conducted for milk in Canada. Over a 20-year period from 1991 to 2011, fat and protein-corrected milk (FPCM; kg/cow/year) increased by 43%, while enteric methane (kg CO_2/kg FPCM) and total emission intensity (kg CO_2/kg FPCM) were decreased by 22% (Jayasundara and Wagner-Riddle, 2014). Similarly, Pelletier et al. (2017) examined the environmental footprint of the egg industry from 1962 – 2012 and reported a decrease in industry total GHG emissions were 57% lower while energy, land and water use decreased by 10%, 71% and 53% lower, respectively.

Improvements in emission intensities in all livestock sectors has occurred as a result of improvements both in animal productivity (reproductive efficiency, weaning weight, carcass weight) and crop yields (barley grain, barley silage, corn grain, and corn silage) as well as irrigation efficiency (Legesse et al., 2016). Production intensity and emission intensity are inversely related, therefore use of precision technologies in livestock production systems can improve sustainability.

An additional outcome of these studies is an examination of the use of human-edible vs non-edible ingredients in livestock diets. Legesse et al. (2016) estimated that approximately 80% of the feedstuffs that cattle in Canada consume in their lifetime are forage-based. Much of this forage is produced on pasture which comprises nearly one-third of all the agricultural land in Canada and is often not suitable for crop production. These values are consistent with those reported on a global basis where approximately 86% of global livestock feed dry matter intake consists of feed materials that are not suitable for human consumption and 57% of land used for feed production is not suitable for food production (Mottet et al., 2018).

Region of production can also yield significant differences in emissions per unit of output as there is significant global variation between developing and developed regions in net emissions and in emissions for specific commodities including milk (Gerber et al., 2011). Milk produced in Canada has a footprint of 0.92 kg CO_2e/kg milk while the global average is 2.5 kg CO_2e/kg milk.
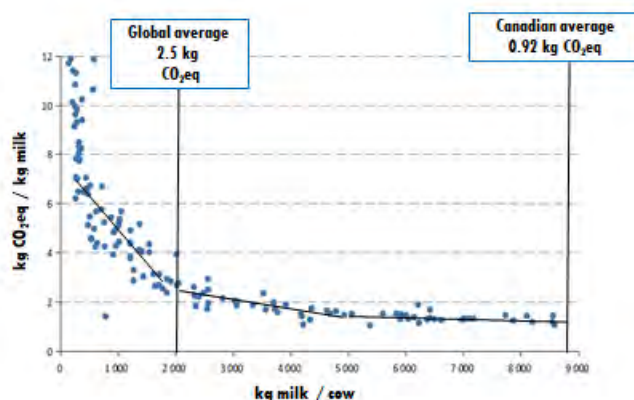
Figure 3: Average greenhouse gas emissions associated with milk production globally and in Canada

Complexity of agroecosystems

Most often, environmental footprints examine only one or two sustainability indices associated with these complex agro-ecosystems as it is extremely challenging to establish a single value to assess overall sustainability. Elements of livestock production systems including carbon sequestration, biodiversity and other ecosystem services, are metrics that are often overlooked in life cycle analysis and footprinting. Indeed, the sustainability of these diverse and multi-functional systems and their role in a circular bio-economy (Figure 4) are difficult to measure and to communicate to the general public.
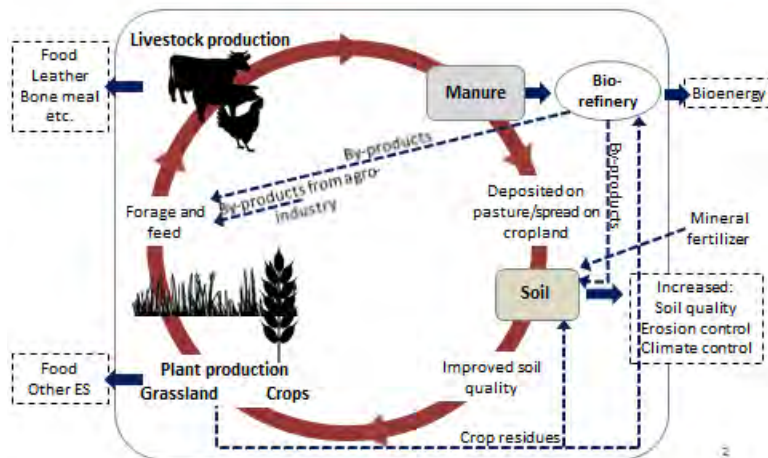
Figure 4. Livestock and sustainable agriculture: the circular economy

As a result of the inherent complexity of livestock production systems, the implications of removal of livestock from the landscape are not readily apparent. Animal-based food products contribute 18% of global calories and account for 25% of protein consumption by humans (FAO STAT, 2016), in addition to providing a variety of micro-nutrients. The FAO has identified an inverse relationship between consumption of animal-sourced foods (g/day) and human nutrition expressed as the global hunger index (FAO, 2018). A small decrease in emissions associated with the simulated removal of livestock from the landscape in the US (White and Hall, 2017) led to a greater excess of dietary energy and increased dietary deficiencies. Similarly, respondents who reported less meat and dairy or no meat and dairy in a 24-hr recall study in France, consumed less protein and several micronutrients, possible increasing the risk of deficiencies (Seves et al., 2017).

From a global perspective, the role of livestock extends beyond nutrition, having social, economic, cultural and political implications in developing countries (Riethmuller, 2003). Not only do they provide essential nutrients for early childhood cognitive development, livestock have the potential to be "transformative" providing cash necessary for food staples, farm inputs, and education, as well as draft power and manure as a fertilizer (Smith et al, 2013).

In addition to diet selection, as consumers, we all have a role to play regarding the environmental impact associated with food waste. Globally, food waste and loss is staggering – with losses of 30% for cereal foods, 45% for fruits and vegetables, 20% for oilseeds and pulses, 45% for roots and tubers, 20% for dairy, 30% for fish and seafood, and 20% for meat (Mottet, 2019). In Canada, total avoidable and unavoidable annual waste along the food value chain is approximately 35.5 million metric tonnes, 32% of which is avoidable and valued at $49.5 billion. This represented 51.8% of the money Canadians spend on food, 3% of Canada's 2016 GDP and enough food to sustain every person in Canada for almost 5 months (Gooch et al., 2019).

Strategies for engagement with consumers

As stakeholders in the livestock sector, we are eager to share our knowledge with consumers. How we capture their attention is an ever-allusive challenge. Engagement between industry stakeholders and consumers in Canada has been facilitated through provincial programs including Agriculture in the Classroom, Open Farm Day, as well as national initiatives including the Canadian Centre for Food Integrity whose mandate is to coordinate research, dialogue, resources and training in Canada's food system. Although sustainable production systems and diets are important for human and environmental well-being, there is no silver bullet approach to define the trade-offs that exist between environmental health, human health, economic feasibility and cultural appropriateness of the Canadian diet. There is a need for dieticians, environmental/agro-ecosystem scientists and policy makers to work together to inform public education and policy initiatives using science-based information to ensure nutrient adequacy, improve health and ensure the environmental sustainability of Canadian diets. However, as we support consumers in their quest to make informed choices regarding diet, we must be mindful there is room in the marketplace for a variety of production systems.

References:

Alexandratos, N. and Bruinsma, J. 2012. World Agriculture Towards 2030/2050: the 2012 revision. ESA Working Paper No. 12-03. Agricultural Development Economics Division, FAO.

Basarab, J., V. Baron, O. Lopez-Campos, J. Aalhus, K. Haugen-Kozyra and E. Okine. 2012. Greenhouse gas emissions from calf- and yearling-fed beef production systems with and without the use of growth promotants. Animals 2:195-220.

Brunn Werner, L., A. Flysjo and T. Tholstrup. 2014. Food and Nutrition Research 58:20687

Capper, J.L. 2011. The environmental impact of United States beef production: 1977 compared with 2007. J. Anim. Sci. 89:4249-4261.

Capper, J. L. and D. J. Hayes. 2012. The environmental and economic impact of removing growth-enhancing technologies from U.S. beef production. J. Anim. Sci. 90:3527-3537.

Charlebois, S., S. Somogyi, J. Music, and I. Caron. 2020. Planet, ethics, health and the new world order in proteins. J. Ag. Studies. 8:171-192.

Drewnowski. A., C. Rehm., A. Martin, E. Verger, M. Voinnesson and P. Imbert. 2015. Energy and nutrient density of foods in relation to their carbon footprint. Am. J. Clin. Nutr. 101:184-191.

Environment and Climate Change Canada. 2020. National Inventory Report - Greenhouse gas sources and sinks in Canada: Executive summary. https://www.canada.ca/en/environment-climate-change/services/climate-change/greenhouse-gas-emissions/sources-sinks-executive-summary-2020.html

Food and Agricultural Organization of the United Nations. 2010. Sustainable diets and biodiversity. Directions and Solutions for Policy, Research and Actions. Rome, FAO

Food and Agricultural Organization of the United Nations. 2018. The state of food security and nutrition in the world. Building climate resilience for food security and nutrition. Rome, FAO.

FAO STAT, 2016. Statistics Division, Food and Agricultural Organization of the United Nations. Rome, FAO.

Gerber, P.J., T. Vellinga, C. Opio, and H. Steinfeld. 2011. Productivity gains and greenhouse gas intensity in dairy systems. Livestock Sci. 139:100-108.

Gooch, M., D. Bucknell, D. LaPlain, B. Dent, P. Whitehead, A. Felfel, L. Nikkel, M. Maguire, M. 2019. The Avoidable Crisis of Food Waste: Technical Report; Value Chain Management International and Second Harvest; Ontario, Canada.

Jayasundara, J and C. Wagner-Riddle, 2014. Greenhouse gas emissions intensity of Ontario milk production in 2011 compared with 1991. Can. J. Anim. Sci. 94:155-173.

Legesse, G., K. Beauchemin, K. Ominski, E. McGeough, R. Kroebel, D. MacDonald, S. Little, and T. McAllister. 2016. Greenhouse gas production of Canadian beef production in 1981 vs 2011. Anim. Prod. Sci. 56:153-168.

Legesse, G., M Cordeiro, K. Ominski, K. Beachemin, R. Kroebel, E. McGeough, S. Pogue and T. McAllister. 2018. Water use intensity of Canadian beef production in 1981 as compared to 2011. Sci. Total Env. 619-620:1030-1039.

Mottet, A. 2019. Perspectives on Sustainable Diets. Dairy Farmers of Canada Symposium. October 29, 2019.

Mottet, A., F. Teillard, P. Boettcher, G. De' Besi and B. Besbes. 2018. Review: Domestic herbivores and food security: current contribution, trends and challenges for a sustainable development. Animal 12:s188-s198.

Mottet, A., C. de Haan, A. Falcucci, G. Tempio, C. Opio, and P. Gerber. 2017. Livestock: On our plates or eating at our table? Global Food Security 14:1-18.

OECD-FAO. 2019. OECD-FAO Agricultural Outlook - Edition 2019. OECD Agriculture Statistics (database), https://doi.org/10.1787/eed409b4-en (accessed on 10 May 2020).

Riethmuller, P. 2003. The social impact of livestock: A developing country perspective. Anim. Sci. J. 74:245-253.

Pelletier, N. 2017. Changes in the life cycle environmental footprint of egg production in Canada from 1962-2012. J. Cleaner. Prod. DOI: 10.1016/j.jclepro.2017.11.212

Rooke, J., J. Flockhart, and N. Sparks, N. 2010. The potential for increasing the concentrations of micro-nutrients relevant to human nutrition in meat, milk and eggs. J. Agric. Sci. 148:603-614.

Seves, S., J. Verkaik-Kloosterman, S. Biesbroek and E. Temme. 2017. Are more environmentally sustainable diets with less meat and dairy nutritionally adequate? Public Health Nutrition. 20: 2050–2062.

Smith, J., K. Sones, D. Grace, S. MacMillan, S. Tarawaii and M. Herrero. 2013. Beyond milk, meat and eggs: Role of livestock in food and nutritional security. Anim. Frontiers 3:6-13.

Tilman, D. and M. Clark. 2014. Global diets link environmental sustainability and human health. Nature 515(7528):518-522

United Nations – World Population Prospects: Highlights. 2019. Department of Economic and Social Affairs, Population Division. (ST/ESA/SER.A/423).

White, R. and M. Hall. 2017. Nutritional and greenhouse gas impacts of removing animals from U.S. agriculture. PNAS: E10301-E10308.

Vieux, F., L. Soler, D. Touazi, N. Darmon. 2013. High nutritional quality is not associated with low greenhouse gas emissions in self-selected diets of French adults. Am. J. Clin. Nutr. 97: 569-583.

Veeramani, A., G. Dias, S. Kirkpatrick. 2017. Carbon footprint of dietary patterns in Ontario, Canada: A case study based on actual food consumption J. Cleaner Prod. 162:1398-1406.

Willett, W., J. Rockström, B. Loken, M. Springmann, T.Lang, S. Vermeulen, T. Garnett, D. Tilman, F. DeClerck, A. Wood, M. Jonell, M. Clark, L. Gordon, J. Fanzo, C. Hawkes, R. Zurayk, J. Rivera, W. De Vries, L. Sibanda, A. Afshin, A. Chaudhary, M. Herrero, R. Agustina, F. Branca, A. Lartey, S. Fan, B. Crona, E. Fox, V. Bignet, M. Troell, T, Lindahl, S. Singh, S. Cornell, K. Reddy, S. Narain, S. Nishtar, C. Murray. 2019. Food in the Anthropocene: the EAT–Lancet Commission on healthy diets from sustainable food systems. Lancet. 399:447-492.

Williamson, C., R. Foster, S. Stanner, S. and J. Buttriss. 2005. Red meat in the diet. Nutrition Bulletin 30:323-355.

Diet for healthy gut:
The secret for overall health and productivity of dairy cattle

L'alimentation pour une bonne santé intestinale : le secret de la santé et de la productivité globales des bovins laitiers

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Abstract

High-producing cattle have increased demands for energy and nutrients. This is the main reason why feeding programs for cattle encourage the use of diets rich in grains and easily fermentable by-products. This feeding leads to a large production of short-chain fatty acids, which supply large amounts of energy and other metabolic substrates to the host, supporting high milk yields, and enhancing cost efficiency of cattle production. However, the inability to absorb large amounts of acids disrupts the homeostatic rumen acid-base regulation, which is a critical for maintaining healthy and optimal conditions for the microbiome to thrive (e.g. normobiosis). As a consequence, the rumen loses some of its main metabolic functions and a large amount of ingested substrates bypasses the rumen undigested and challenges the normobiosis in the lower parts of the digestive tract. Accumulating evidence suggests that dysbiosis leads to a disruption of homeostasis and development of inflammation in cattle. Most importantly, this disorder impairs rumen functioning and exacerbates health status of animals, triggering cascades of events that lead to many metabolic and infectious diseases such as laminitis, ketosis, milk fat depression syndrome, displaced abomasum, hindgut acidosis, shedding of pathogens, systemic inflammation and poor reproduction. This article will deal with challenges to maintain proper rumen and gut health in high-producing cattle, highlighting current data of rumen microbiome and metabolome research. This article will also show underlying mechanisms of gut health disorders and provide dietary recommendations to maintain and enhance gut health in high yielding dairy cows.

Résumé

Les bovins à rendement élevé ont des besoins accrus en énergie et en nutriments. C'est la raison principale pour laquelle les programmes alimentaires des bovins encouragent l'utilisation de régimes riches en céréales et en sous-produits facilement fermentescibles. Cette alimentation entraîne une production importante d'acides gras à chaîne courte qui fournissent de grandes quantités d'énergie et d'autres substrats métaboliques à l'hôte, une situation qui favorise les rendements laitiers élevés et améliore la rentabilité de la production bovine. Toutefois, l'incapacité à absorber de grandes quantités d'acides perturbe la régulation homéostatique acide-base du rumen, un mécanisme essentiel au maintien de conditions saines et optimales assurant le bon développement du microbiome (par exemple, la normobiose). En conséquence, le rumen perd certaines de ses principales fonctions métaboliques, et une grande partie du substrat ingéré échappe à la dégradation dans le rumen, perturbant la normobiose plus loin dans le tube digestif. Des preuves de plus en plus nombreuses suggèrent que la dysbiose entraîne une perturbation de l'homéostasie et le développement d'une inflammation chez les bovins. Plus important encore, ce trouble altère le fonctionnement du rumen et aggrave l'état de santé des animaux, déclenchant des cascades d'événements qui conduisent à de nombreuses maladies métaboliques et infectieuses, telles que la fourbure, l'acétonémie, le syndrome de dépression des matières grasses du lait, le déplacement de la caillette, l'acidose au niveau de l'intestin postérieur, l'excrétion d'agents pathogènes, l'inflammation systémique et les difficultés de reproduction. Cet article traite des défis à relever pour maintenir la bonne santé du rumen et de l'intestin chez les bovins à rendement élevé, en insistant sur les données actuelles de la recherche sur le microbiome et le métabolome du rumen. Cet article présente également les mécanismes sous-jacents des troubles de la santé intestinale et fournit des recommandations nutritionnelles pour maintenir et améliorer la santé intestinale des vaches laitières à haut rendement.

Introduction

Gut health is intrinsically linked with animal health, which in turn dictates cost efficient production. Animal health and productivity are intrinsically linked, as are gut health and animal health. In many diseases, diet is implicated as a contributing factor by having direct effects on host metabolism, immune responses, and microbiome composition, subsequently altering disease susceptibility (Plaizier et al. 2018). Gut health is defined as the balance of homeostasis between host and microbiome (normobiosis) as well as the resistance to external and endogenous disturbances (Lozupone et al. 2012). Dysbiosis is a state in which the microbiota produces harmful effects via changes in relative abundance, changes in metabolic activities or changes in diversity and distribution (Garcia et al. 2017). The strongest disturbances relate to diet, including differences in form, composition, dry matter intake (DMI), the application of antibiotics, or addition of transient bacteria through feed additives or water. Gut homeostasis is maintained when the diet meets the needs of the commensal microbiota, the dynamic barrier function and tolerant immune responses of the host (Khafipour et al. 2016; Steele et al. 2016). This dynamic network, when in balance, provides a symbiotic, and mutually beneficial relationship (Plaizier et al. 2018). This relationship is especially unique in foregut fermenters, where the microbial ecosystem utilizes nutrients unavailable to the host to produce short chain fatty acids (SCFA) and microbial protein, which the cow can then metabolize for milk production (Russell and Rychlik, 2001). In this regard, when feeding cows, we are actually feeding the microbes (Neubauer et al. 2018). Therefore,

feeding management is not only crucial for the metabolic adaptation for high production dairy cows, but for the balance of the rumen microbial ecosystem as well. When the symbiotic relationship in the rumen is disturbed, rumen health becomes a systemic concern. Besides the luminal environment, the integrity and health status of the rumen epithelium and gastrointestinal mucosa is also a very important factor in gut health. In addition to their central metabolic roles, rumen epithelial cells are the first line of defense against hostile rumen conditions such as acidic pH, high osmotic pressure, and harmful microbial-derived metabolites (Chen et al. 2012).

Challenges to Maintaining Gut Health and Normobiosis in Dairy Cows

One major gut health challenge is the prioritization of gluconeogenesis for milk production. Especially in early lactation when metabolic demand is high and insufficient dietary precursors for glucose production are provided, the body will mobilize tissue resulting in ketosis and fatty liver disease (Zebeli et al. 2015). In an attempt to prevent metabolic disorders due to a negative energy balance, it is common to provide high energy, rapidly fermentable diets to increase the energy availability at a time when dry matter intake (DMI) is often limited (Zebeli et al. 2015). In practice this means concentrates are fed, often at the expense of fibre-rich forages, which can cause major imbalances in the acid-base balance by reducing salivary buffering contribution to the rumen through reductions physically effective neutral detergent fibre (peNDF; Zebeli et al. 2012; Figure 1). These changes result in a cascade of changes within the microbial ecosystem that can have a negative impact on rumen and animal health. Also problematic is the increase in feed intake in high producing dairy cows around peak lactation. This increase in fermentable substrate results in an increase in rumen fermentation and a larger pool of VFAs for rumen buffering. Since chewing time is reduced in diets with high grain, low forage content, the salivary contribution to ruminal buffering is also decreased (Beauchemin, 2018). Again, this increase in VFA results in lowered pH and gastrointestinal dysbiosis (Enemark, 2008).

Another major challenge to maintaining gut health is the impact of diet-induced dysbiosis on secondary disease susceptibility. When disturbances occur, functional adaptations away from growth towards cellular pH stabilization by more sensitive members of the gastrointestinal microbial ecosystem may decrease relative abundances in the community (Russell and Dombrowski, 1980) and impair efficient nutrient utilization (Russell and Wilson, 1996). This, in turn, results in the increased flow of nutrients to the hindgut from the rumen causing further disturbances in gastrointestinal microbiota (Gressley et al. 2011). Increased nutrient flow increases hindgut fermentation, reducing digesta pH, resulting in diarrhea (Li et al. 2012). Alterations in pH, osmolality, and the microbial community structure throughout the gastrointestinal tract impact absorptive and barrier functions of the epithelia causing inflammation (Khiaosa-ard and Zebeli, 2014). Hindgut acidosis is therefore similar to SARA and can also disrupt nutrient utilization, impair the gastrointestinal microbiota, reduce the absorptive and barrier capacities of the epithelia, and trigger inflammatory responses (Plaizier et al. 2018).

Dietary disturbances can result in localized inflammation, tissue damage effecting barrier function, and the infiltration of pathogens into the peripheral blood circulation (Kleen et al. 2003; Plaizier et al. 2008; Figure 1). Increased stress on the gastrointestinal microbes, as a result of an accumulation of protons, results in microbial cell death for pH sensitive microbes, and the release

of microbial-derived toxic compounds into the digestive milieu including lipopolysaccharides (LPS) and biogenic amines (Ametaj et al. 2010; Aschenbach et al. 2011; Humer et al. 2018a). As a result, lowered pH and the increase in LPS also causes disruptions to the mucosa barrier function of the gastrointestinal tract (Aschenbach and Gäbel, 2000), offering the opportunity for microbe-derived toxic compounds to translocate into the systemic circulation. This event could lead to systemic inflammation and repeated bouts of dysbiosis may reduce immune responsiveness due to exhaustion from repeated exposure to systemic endotoxins and possibly opportunistic gastrointestinal pathogens. A derailment of metabolic function and immune response can result in an increased incidence rate for other diseases including laminitis (Nocek, 1997), displaced abomasum (LeBlanc et al. 2005), and liver lipolysis (Ametaj et al. 2010).

Additionally, host variation is an additional challenge to managing gut health, as susceptibility to dysbiosis varies between animals, even within a herd with similar genetics and identical environmental factors (Khafipour et al. 2009; Penner et al. 2009; Petri et al. 2013). Variations in physiological parameters, including previous exposure to lactation diets, as well as social parameters impacting eating behavior are all sources of possible variation within a herd (Figure 1). Variations in individual microbiomes and host epithelial function are also believed to play a role in animal variation (Petri et al. 2019).
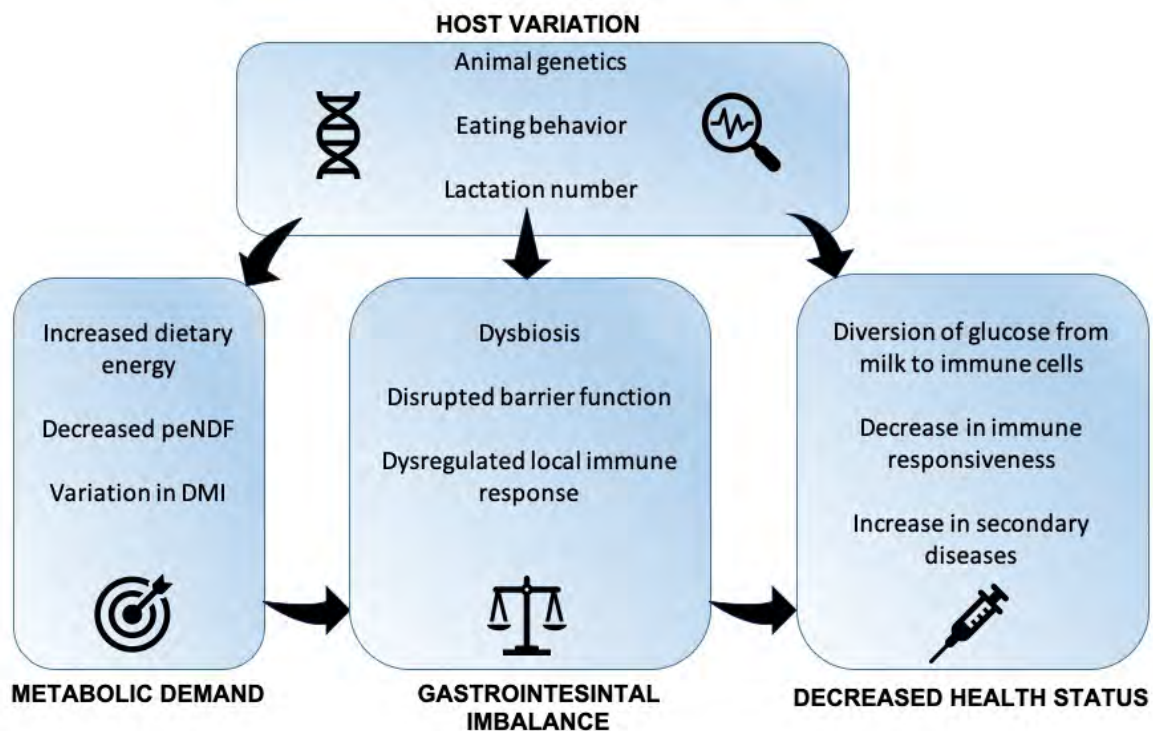
Figure 1. Challenges associated with maintaining gut health in high production dairy cows

Metabolic Adaptations and Underlying Mechanisms in Cows

The rumen microbial ecosystem is a vast community of bacteria, protozoa, archaea and fungi, working in a coordinated manner to optimize nutrient utilization, exemplifying a symbiotic or mutualistic relationship with the host (Garcia et al. 2017). The gastrointestinal microbiome of cattle is no different in its complexity or symbiotic relationship. However, the role of microorganisms in other segments of the gastrointestinal tract (GIT), such as the small and large intestine, have received comparatively little attention (Mao et al. 2015). Members of gut microbiota differ in their functionality and ability to utilize different groups of substrates (Henderson et al. 2015; Mao et al. 2015; Petri et al. 2017). Hence, a higher richness and diversity of microbiota is beneficial as it enhances the stability and often enables a more efficient use of nutrient resources (Russell and Rychlik, 2001; Ley et al. 2006). However, abiotic stresses including those induced by high grain feeding, can temporarily or permanently change the composition and functionality of the microbiota. These changes include reduced richness, evenness and diversity of microbiota, as well as a reduction in the abundance of many beneficial microbial taxa in both the reticulo-rumen (Khafipour et al. 2009; Mao et al. 2013, 2015; Petri et al. 2013) and in the hindgut (Mao et al. 2015; Plaizier et al. 2017). Specific changes in the ruminal abundances of lactic acid producing and lactic acid utilizing species, and reductions in the relative abundances of fibrolytic species are most commonly documented in association with highly fermentable diets (Petri et al. 2013; Plaizier et al. 2017). Similarly, in the hindgut, increased starch feeding may increase rumen bypass and as a result the composition and functionality of the hindgut microbiota are shifted. During a state of dysbiosis, the microbiota is prone to invasion and overgrowths of pathogens exploiting the niches that are left open after disturbances. Changes to the populations of pathogenic bacterium in the rumen and gastrointestinal tract such as *Escherichia coli* and *Streptococcus bovis* are often associated with the disruption of ecological balance within the ecosystem caused by highly fermentable diets (Khafipour et al. 2009; Petri et al. 2013). The overgrowth and invasion of *Fusobacterium necrophorum* resulting in liver abscesses is an example of a potential trigger of disease in cattle fed high grain diets resulting in damage to the rumen epithelium (Nagaraja and Chengappa, 1998). While it is clear that the changes in the microbial populations leading to an overgrowth of pathogenic microbes promotes disease development, the mechanisms involved are less clear.

Microbial Metabolites
Microbes, including the commensals, produce an array of products that include metabolites and parts of their cell membranes known as microorganism-associated molecular patterns (MAMP; Garcia et al. 2017). Most conventional studies investigating the effect of low pH and high grain diets on the metabolism of cows have focused on single metabolites (Zebeli et al. 2011) such as LPS (Nagaraja et al. 1978). However, metabolomics technology has become an important part of livestock science to better understand health and disease. Using metabolomics, Ametaj et al. (2010) and Saleem et al. (2012) performed comprehensive studies characterizing the bovine rumen fluid metabolome, reporting potentially toxic biomarkers in the rumen fluid of dairy cows that experienced ruminal dysbiosis after being fed barley-rich diets. Humer et al. (2018a) looked at the metabolomic profile of cows fed either an all forage or a high-concentrate diet and found negative relationships between rumen LPS and biogenic amines and blood metabolites including amino acids, phosphatidylcholines and sphingomyelin. This indicates an impact of microbial metabolites on host metabolism and function. Such microbial-derived toxic compounds have been long suggested as biomarkers of dysbiosis (Plaizier et al. 2012), but the mode of action is not completely

understood. However, from studies in other mammals, it is clear that frequent perturbations of the normal microbial population in the GIT can lead to an inappropriate activation of the host immune system, promoting chronic inflammation and subsequent disease (Carney, 2016; Garcia et al. 2017). It has been suggested that endotoxins are part of immune regulation mechanisms that prevent excessive inflammation, when the costs may exceed the benefits. However, this tolerance means immune responses are either limited or absent, which may result in increased susceptibility to secondary infections. Gott et al. (2015) observed grain induced ruminal dysbiosis reduced acute phase proteins (serum amyloid A) in milk after intra-mammary challenge with *E. coli* LPS, which could theoretically make cows more susceptible to mastitis associated pathogens.

Host gene expression
The GIT is the largest interface between the animal and the environment, not only is it responsible for facilitating nutrient uptake by the host, but it also functions as a barrier preventing the uptake of harmful microbes and some harmful products (Farhadi et al. 2003; Garcia et al. 2017). The stratified squamous structure of the ruminal epithelium appears to have evolved to deal with the abrasive feed and large microbial population colonizing the rumen and is more adapted to lower ruminal pH (Garcia et al. 2017). The hindgut is lacking this stratified structure and instead has a protective mucous layer (Steele et al. 2016). Furthermore, the mechanisms employed by the epithelial structures in the rumen and mid or hindgut differ based on these dramatically different epithelial structures. Specifically, specialized cells (goblet, paneth and M cells) are not present in the foregut, and the rumen epithelium and lamina propria lack organized lymphoid tissues (Garcia et al. 2017). Some studies have associated ruminal epithelium damage with compromised rumen integrity in cattle fed high grain diets, which may enable the translocation of microbes and their products to the bloodstream (Devant et al. 2016; Garcia et al. 2017). Khafipour et al. (2009) postulated that the translocation of pathogenic organisms and their metabolites from the GIT into systemic circulation is possibly occurring from the hindgut as opposed to the rumen based on structural design. The lack of stratified squamous structure would possibly make the hindgut more sensitive to low pH associated with hindgut dysbiosis. Although this is still believed to be true, the mechanisms and mode of action for these events remains largely undocumented and requires further research.

Advances in technology over the last decade have enhanced our understanding of the microbiome's role in regulating host gene expression. We now understand how activation of specific genes coding for key molecular compounds results in immune activation during dysbiosis, systemic inflammation and other metabolic disturbances of the host, as well as a lowered mucosal capability for absorption and metabolism of SCFA (Penner et al. 2009; 2011; Aschenbach et al. 2011). Numerous studies have looked at the response of pattern recognition receptors (PRR) in the rumen in response to dietary induced dysbiosis (Minuti et al. 2015; McCann et al. 2016; Petri et al. 2018; 2019) with varying success. Recognition of a mucosal associated molecular pattern (MAMP) by a toll-like receptor (TLR) complex leads to the activation of inflammatory mediators (Garcia et al. 2017). Several studies have reported the expression of TLR in ruminal tissue and the potential to response to ruminal LPS (Chen et al. 2012; Minuti et al. 2015). And though multiple studies have reported associations between ruminal LPS concentrations, and its translocation into circulation (Khafipour et al. 2009; Humer et al. 2018a), the mechanisms by which this occurs are not fully understood. Postulated mechanisms include passive diffusion through the damaged rumen epithelium or via the lower gut, due to disruptions in the intestinal tight junctions (Turner,

2009; Garcia et al. 2017). High production and or reduced absorption of ruminal VFA may also induce increments in osmotic pressure, disturb Na transport, and impair ruminal barrier function, further increasing the risk of microbial and microbial-derived compound translocation (Owens et al. 1998; Humer et al. 2018; Petri et al. 2019). Regardless of whether or not translocation occurs via rumen or lower gut, microbes and or microbe-derived compounds can rapidly enter the portal vein and be taken up in the liver, increasing the changes of systemic inflammation and secondary infections (Andersen et al. 1994).

Dietary Strategies to Manage Gut Health in Transition Cows

With the push for reduced usage of antibiotics in animal production, it is critical that a combination of alternative strategies be used to manage gut health. Gastrointestinal dysbiosis due to high grain feeding may be attenuated by a variety of strategies. The guiding principle is management of diet during the early and mid-lactation period as an important factor influencing gut health, subsequently milk production, and metabolic disorder incidence in high production cows. The possible approaches that can be taken are:

1) Feed management inclusive peNDF content, and adaptation periods
2) Supplementation with feed additives inclusive phytogenic compounds, probiotics/direct-fed microbials (DFMs), and yeast derived products

Feeding Management
The feeding management principles for mitigating dysbiosis in high production cows should aim to maintain acid-base regulation in the rumen so that the production of SCFA does not overwhelm saliva and epithelial buffering mechanisms (Aschenbach et al. 2011). The ingestion of large meals in a short time predisposes cows to rumen disorders because rumen pH decreases following meals in general and the rate of decrease in rumen pH is high when a meal is large (Krause and Oetzel, 2006). This is explained by the reduced salivary secretion when dairy cows ingest a large meal in a short time, resulting in a decrease in the buffering capacity of the rumen and a consequent depression in the rumen pH (Beauchemin et al. 2008). Therefore, to maximize the buffering capacity of the rumen and lowering the episodes of low rumen pH, dairy cows should be managed so as to consume their diet slowly and more frequently in small meals during the day. Although the feeding of total mixed rations (TMR) aims to ensure the adequate ingestion of peNDF and minimize the selective consumption of grains or fine particles, these sorting behaviours still occur (Armentano and Pereira, 1997; DeVries et al. 2008). Management of these behaviours can be achieved by distributing the TMR more frequently, providing sufficient eating space, avoiding stress and adequately mixing the feed. Diets low in peNDF and in excess of rumen degradable starch (RDS) should also be avoided as this will be converted to lactic acid and can drastically reduce rumen pH (Nagaraja and Titgemeyer, 2007). Though exact recommendations of particle size vary based on composition of the diet and feeding management, peNDF is best management with regular measurement of both TMR and the orts will provide the most accurate estimation of particle size distribution based on various dietary composition and the associated sorting behaviours (Humer et al. 2018b). In addition, by providing adequate fiber and particle length (Zebeli et al. 2012) and >30% NDF for adequate production of microbial protein, which is the primary source of amino acids for the cow (Humer et al. 2018b). Along with a consistency in the

supply of feed, these feed management components are important in the prevention of ruminal dysbiosis, as they are most often adjusted in used in studies trying to invoke a dysbiotic state (Nagaraja and Titgemeyer, 2007). Monitoring rumination activity is another feed related management strategy as research suggests that cows with a greater risk of developing rumen disorders have a slower increase in rumination time after calving (Calamari et al. 2014).

Although nutritional management practices can reduce the incidence of dysbiosis, some animals are more susceptible to the effects of a high-grain diet than others (Brown et al. 2000; Bevans et al. 2005; Penner et al. 2009; Petri et al. 2013). Feeding strategies for high producing cows should target adaptation of the rumen epithelium and the microbiome to the large amounts RDS and decreases in forage content to maintain the balance between production and absorption of SCFA (Kleen et al. 2003; Zebeli et al. 2015). Humer et al. (2015) showed longer periods of time below pH 5.8 and 6.0 in primiparous cows compared to those in second or higher lactation and postulated that those cows possible had fewer papillae and different microbial community profiles compared to mature cows (Penner et al. 2007; Bramley et al. 2008). Therefore, this adaptation is particularly important and in primiparous cows which have a higher susceptibility to disbalance as a result of physiological naivety. Recent studies have shown that physiological adaption of the rumen epithelium to changes in diet requires at least 14 days (Petri et al. 2019) and would be beneficial up to 4 - 6 weeks (Bannink et al. 2012; Zebeli et al. 2015). The length of recovery of the microbiome and epithelial microbiota and host gene expression from a dysbiosis challenge shows a similar adaptation time (Wetzel et al. 2017; Petri et al. 2019). This would imply that for at risk animals, such as those that are naïve to high-grain diets, it is of special importance to optimize the above feeding and management parameters.

Supplementation with Feed Additives
In light of these feed management strategies, it can still be difficult to achieve a balance between feeding readily fermentable carbohydrates to meet the high energy levels required for productivity and to minimize adverse health risks that result from feeding starches and sugars. Recently there has been increasing research in the usage of feed additives for the stabilization and recovery of rumen homeostasis. Current control strategies are unlikely to manage ruminal dysbiosis in the entire herd, but in combination with feed additive strategies, may significantly decrease the risk of nutrition related diseases.

Sodium bicarbonate and magnesium oxide are commonly used rumen buffers (Staples and Lough, 1989; Golder, 2014). By affecting the dietary cation-anion difference, bicarbonates and buffers might prevent an overgrowth of acid tolerant, lactic acid bacteria by preventing pH depression. However, literature on the effects of feeding buffers on ruminal pH are inconsistent and generally are used only as a supportive measure due to their minimal effects on the overall acid-base balance (Krause and Oetzel, 2006).

Similar to rumen buffers, the supplementation with lactate-utilizing microorganisms has been investigated to reduce the impact of lactic acid production when high grain diets are fed. A number of products of single or mixed bacterial cultures are used in cattle, including strains from *Bifidobacterium*, *Enterobacteria*, *Streptococcus*, *Prevotella*, *Bacillus*, *Lactobacillus*, *Megasphaera*, and *Propionibacterium spp.* have been used in the dairy industry in different stages of production (McAllister et al. 2011). However, responses are inconsistent and reflecting

differences in inclusion level, diet, feeding management and other factors (Humer et al. 2018b). There is often an increase in milk production as a result of supplementing DFMs, increased health and performance in calves and reduced SARA (Krehbiel et al. 2003). Direct-fed microbials have been shown to decrease the time and duration spent at low pH, increase propionate concentrations, and maintain community diversity (Krehbiel et al. 2003). Although there has been a substantial amount of work on establishing the mechanism, the evidence is limited.

Despite some contradictory results, the supplementation of feed additives including yeast, enzymes, and phytogenic compounds show modest effects on the support and recovery of the rumen homeostasis in cattle fed high grain diets. Live yeasts have been shown to increase rumen pH, reduce lactic acid, increase fibre digestion and increase SCFA production (Chaucheyras-Durand et al. 2008). Yeast fermentation products have shown increased chewing time (Kröger et al. 2017), increased DMI, and stabilization of the rumen under SARA conditions (Neubauer et al. 2018). Similar results have been seen for phytogenic compounds showing effects of the rumen pH (Kröger et al. 2017) as well as butyrate concentrations, and the proportions of rumen microbes (Neubauer et al. 2019). While mode of action is generally not understood, it is postulated that supplementation may delay the onset of VFA accumulation. However, this and further research regarding the possible synergistic properties of these feed additives in preventing gastrointestinal dysbiosis and other related diseases is still needed.

Conclusions

Understanding the mechanisms resulting in animal variation in susceptibility to dysbiosis and conditions such as SARA remains a primary concern in the prevention of metabolic diseases and the improvement of animal gut health and production. The advancement of 'omics technology is continually providing insights into the host-microbiome interactions, as well as the potential mode of action for feed supplementation products. However, the use of this knowledge for the identification of dysbiosis susceptibility or intervention methods is still a ways off. Therefore, a combination of measures including feed management, adaptation periods, and assessment of chewing and sorting behavior will be required assessing and improving gut health. In addition, under periods of metabolic adaptation, and during high grain feeding, feed supplements can be used to reduce dysbiosis and supporting gut homeostasis.

References

Armentano, L., and M. Pereira, M. 1997. Measuring the Effectiveness of Fiber by Animal Response Trials. J. Dairy Sci., 80(7), 1416–1425.

Ametaj, B. N., Zebeli, Q., Saleem, F., Psychogios, N., Lewis, M. J., Dunn, S. M., Xia, J., and D. S Wishart. 2010. Metabolomics reveals unhealthy alterations in rumen metabolism with increased proportion of cereal grain in the diet of dairy cows. Metabolomics, 6(4), 583–594.

Andersen, P. H., Hesselholt, M., and N. Jarløv. 1994. Endotoxin and arachidonic acid metabolites in portal, hepatic and arterial blood of cattle with acute ruminal acidosis. Acta Vet. Scand., 35(3), 223-234.

Aschenbach, J. R., and G. Gäbel. 2000. Effect and absorption of histamine in sheep rumen: significance of acidotic epithelial damage. J. Anim. Sci., 78(2), 464.

Aschenbach, J. R., Penner, G. B., Stumpff, F., and G. Gäbel. 2011. RUMINANT NUTRITION SYMPOSIUM: Role of fermentation acid absorption in the regulation of ruminal pH12. J. Anim. Sci., 89(4), 1092–1107.

Bannink, A., Gerrits, W. J. J., France, J., and J. Dijkstra. 2012. Variation in rumen fermentation and the rumen wall during the transition period in dairy cows. Anim. Feed Sci. Tech., 172(1-2), 80–94.

Beauchemin, K. A., Eriksen, L., Nørgaard, P., and L. M. Rode. 2008. Salivary secretion during meals in lactating dairy cattle. J. Dairy Sci., *91*(5), 2077-2081.

Beauchemin, K. A.. 2018. Invited review: Current perspectives on eating and rumination activity in dairy cows. J. Dairy Sci., 101(6), 4762–4784.

Bevans, D. W., Beauchemin, K. A., Schwartzkopf-Genswein, K. S., McKinnon, J. J., and T. A. McAllister. 2005. Effect of rapid or gradual grain adaptation on subacute acidosis and feed intake by feedlot cattle1,2. J. Anim. Sci., 83(5), 1116–1132.

Bramley, E., Lean, I. J., Fulkerson, W. J., Stevenson, M. A., Rabiee, A. R., and N. D. Costa. 2008. The Definition of Acidosis in Dairy Herds Predominantly Fed on Pasture and Concentrates. J. Dairy Sci., 91(1), 308–321.

Brown, M. S., Krehbiel, C. R., Galyean, M. L., Remmenga, M. D., Peters, J. P., Hibbard, B., Robinson, J., and W. M. Moseley. 2000. Evaluation of models of acute and subacute acidosis on dry matter intake, ruminal fermentation, blood chemistry, and endocrine profiles of beef steers. J. Anim. Sci., 78(12), 3155.

Calamari, L., Soriani, N., Panella, G., Petrera, F., Minuti, A., and E. Trevisi. 2014. Rumination time around calving: An early signal to detect cows at greater risk of disease. J. Dairy Sci., 97(6), 3635–3647.

Carney, E. F.. 2016. Microbiota trigger inflammation. Nat. Rev. Nephrol., 12(7), 376–376.

Chaucheyras-Durand, F., Walker, N. D., and A. Bach. 2008. Effects of active dry yeasts on the rumen microbial ecosystem: Past, present and future. Anim. Feed Sci. Tech., 145(1-4), 5–26.

Chen, Y., Oba, M., and L. L. Guan. 2012. Variation of bacterial communities and expression of Toll-like receptor genes in the rumen of steers differing in susceptibility to subacute ruminal acidosis. Vet. Microb., 159(3-4), 451–459.

Devant, M., Penner, G. B., Marti, S., Quintana, B., Fábregas, F., Bach, A., and A. Arís. 2016. Behavior and inflammation of the rumen and cecum in Holstein bulls fed high-concentrate diets with different concentrate presentation forms with or without straw supplementation1. J. Anim. Sci., 94(9), 3902–3917.

DeVries, T. J., Dohme, F., and K. A. Beauchemin. 2008. Repeated Ruminal Acidosis Challenges in Lactating Dairy Cows at High and Low Risk for Developing Acidosis: Feed Sorting. J. Dairy Sci., 91(10), 3958–3967.

Enemark, J. M. D.. 2008. The monitoring, prevention and treatment of sub-acute ruminal acidosis (SARA): A review. Open Vet. J., 176(1), 32–43.

Farrhadi, A., Banan, A., Fields, J., and A. Keshavarzian. 2003. Intestinal barrier: An interface between health and disease. J. Gastroenterol. Hepatol., 18(5), 479–497.

Garcia, M., Bradford, B. J., and T. G. Nagaraja. 2017. Invited Review: Ruminal microbes, microbial products, and systemic inflammation 1,2 ARPAS Symposium: Understanding Inflammation and Inflammatory Biomarkers to Improve Animal Performance at the ADSA–ASAS Joint Annual Meeting, Salt Lake City, Utah, July 2016. The Professional Animal Scientist, 33(6), 635–650.

Golder, H. M., Celi, P., Rabiee, A. R., and I. J. Lean. 2014. Effects of feed additives on rumen and blood profiles during a starch and fructose challenge. J. Dairy Sci., 97(2), 985–1004.

Gott, P. N., Hogan, J. S., and W. P. Weiss. 2015. Effects of various starch feeding regimens on responses of dairy cows to intramammary lipopolysaccharide infusion. J. Dairy Sci., 98(3), 1786–1796.

Gressley, T. F., Hall, M. B., and L. E. Armentano. 2011. RUMINANT NUTRITION SYMPOSIUM: Productivity, digestion, and health responses to hindgut acidosis in ruminants1. J. Anim. Sci., 89(4), 1120–1130.

Henderson, G., Cox, F., Ganesh, S., Jonker, A., Young, W., and P. H. Janssen. 2015. Rumen microbial community composition varies with diet and host, but a core microbiome is found across a wide geographical range. Sci. Rep., 5(1).

Humer, E., Khol-Parisini, A., Gruber, L., Gasteiner, J., Abdel-Raheem, S. M., and Q. Zebeli. 2015. Long-term reticuloruminal pH dynamics and markers of liver health in early-lactating cows of various parities fed diets differing in grain processing. J. Dairy Sci., 98(9), 6433–6448.

Humer, E., Kröger, I., Neubauer, V., Schedle, K., Reisinger, N., and Q. Zebeli. 2018a. Supplementing phytogenic compounds or autolyzed yeast modulates ruminal biogenic amines and plasma metabolome in dry cows experiencing subacute ruminal acidosis. J. Dairy Sci., 101(10), 9559–9574.

Humer, E., Petri, R. M., Aschenbach, J. R., Bradford, B. J., Penner, G. B., Tafaj, M., Südekum, K. H. and Q. Zebeli. 2018b. Invited review: Practical feeding management recommendations to mitigate the risk of subacute ruminal acidosis in dairy cattle. J. Dairy Sci., 101(2), 872–888.

Khafipour, E., Li, S., Plaizier, J. C., and D. O. Krause. 2009. Rumen Microbiome Composition Determined Using Two Nutritional Models of Subacute Ruminal Acidosis. Appl. Environ. Microb., 75(22), 7115–7124.

Khafipour, E., Li, S., Tun, H. M., Derakhshani, H., Moossavi, S., and J. C. Plaizier. 2016. Effects of grain feeding on microbiota in the digestive tract of cattle. Anim. Front., 6(2), 13–19.

Khiaosa-Ard, R., and Q. Zebeli. 2014. Cattle's variation in rumen ecology and metabolism and its contributions to feed efficiency. Livest. Sci., *162*, 66-75.

Kleen, J. L., Hooijer, G. A., Rehage, J., and J. P. T. M. Noordhuizen. 2003. Subacute Ruminal Acidosis (SARA): a review. J. Vet. Med. A, 50(8), 406–414.

Krause, K. M., and G. R Oetzel. 2005. Inducing Subacute Ruminal Acidosis in Lactating Dairy Cows. J. Dairy Sci., 88(10), 3633–3639.

Krehbiel, C. R., Rust, S. R., Zhang, G., and S. E. Gilliland. 2003. Bacterial direct-fed microbials in ruminant diets: Performance response and mode of action. J. Anim. Sci., *81*(14_suppl_2), E120-E132.

Kröger, I., Humer, E., Neubauer, V., Reisinger, N., Aditya, S., and Q, Zebeli. 2017. Modulation of chewing behavior and reticular pH in nonlactating cows challenged with concentrate-rich diets supplemented with phytogenic compounds and autolyzed yeast. J. Dairy Sci., 100(12), 9702–9714.

LeBlanc, S. J., Leslie, K. E., and T. F. Duffield. 2005. Metabolic Predictors of Displaced Abomasum in Dairy Cattle. J. Dairy Sci., 88(1), 159–170.

Ley, R. E., Turnbaugh, P. J., Klein, S., and J. I. Gordon. 2006. Human gut microbes associated with obesity. Nature, 444(7122), 1022–1023.

Li, S., Gozho, G. N., Gakhar, N., Khafipour, E., Krause, D. O., and J. C. Plaizier. 2012. Evaluation of diagnostic measures for subacute ruminal acidosis in dairy cows. Can. J. Anim. Sci., 92(3), 353–364.

Lozupone, C. A., Stombaugh, J. I., Gordon, J. I., Jansson, J. K., and R. Knight. 2012. Diversity, stability and resilience of the human gut microbiota. Nature, 489(7415), 220–230.

Mao, S. Y., Zhang, R. Y., Wang, D. S., and W. Y. Zhu. 2013. Impact of subacute ruminal acidosis (SARA) adaptation on rumen microbiota in dairy cattle using pyrosequencing. Anaerobe, 24, 12–19.

Mao, S., Zhang, M., Liu, J., and W. Zhu. 2015. Characterising the bacterial microbiota across the gastrointestinal tracts of dairy cattle: membership and potential function. Sci. Rep., 5(1).

McAllister, T. A., Beauchemin, K. A., Alazzeh, A. Y., Baah, J., Teather, R. M., and K. Stanford. 2011. Review: The use of direct fed microbials to mitigate pathogens and enhance production in cattle. Can. J. Anim. Sci., 91(2), 193–211.

McCann, J. C., Luan, S., Cardoso, F. C., Derakhshani, H., Khafipour, E., and Loor, J. J. (2016). Induction of Subacute Ruminal Acidosis Affects the Ruminal Microbiome and Epithelium. Front. Micro., 7.

Minuti, A., Palladino, A., Khan, M. J., Alqarni, S., Agrawal, A., Piccioli-Capelli, F., Cardoso, F. C., Trevisi, E., and J. J. Loor. 2015. Abundance of ruminal bacteria, epithelial gene expression, and systemic biomarkers of metabolism and inflammation are altered during the peripartal period in dairy cows. J. Dairy Sci., 98(12), 8940–8951.

Nagaraja, T. G., Bartley, E. E., Fina, L. R., and H. D. Anthony. 1978. Relationship of Rumen Gram-Negative Bacteria and Free Endotoxin to Lactic Acidosis in Cattle. J. Anim. Sci., 47(6), 1329–1337.

Nagaraja, T. G., and M. M. Chengappa. 1998. Liver abscesses in feedlot cattle: a review.J. Anim. Sci., 76(1), 287.

Nagaraja, T. G., and E. C. Titgemeyer. 2007. Ruminal Acidosis in Beef Cattle: The Current Microbiological and Nutritional Outlook. J. Dairy Sci., 90, E17–E38.

Neubauer, V., Petri, R., Humer, E., Kröger, I., Mann, E., Reisinger, N., and Q. Zebeli. 2018. High-grain diets supplemented with phytogenic compounds or autolyzed yeast modulate ruminal bacterial community and fermentation in dry cows. J. Dairy Sci., 101(3), 2335–2349.

Nocek, J. E.. 1997. Bovine Acidosis: Implications on Laminitis. J. Dairy Sci., 80(5), 1005–1028.

Owens, F. N., Secrist, D. S., Hill, W. J., and D. R. Gill. 1998. Acidosis in cattle: a review. J. Anim. Sci., 76(1), 275.

Penner, G. B., Beauchemin, K. A., and T. Mutsvangwa. 2007. Severity of Ruminal Acidosis in Primiparous Holstein Cows During the Periparturient Period. J. Dairy Sci., 90(1), 365–375.

Penner, G. B., Aschenbach, J. R., Gäbel, G., Rackwitz, R., and M. Oba. 2009. Epithelial Capacity for Apical Uptake of Short Chain Fatty Acids Is a Key Determinant for Intraruminal pH and the Susceptibility to Subacute Ruminal Acidosis in Sheep. J. Nutri., 139(9), 1714–1720.

Petri, R. M., Schwaiger, T., Penner, G. B., Beauchemin, K. A., Forster, R. J., McKinnon, J. J., and T. A. McAllister. 2013. Characterization of the Core Rumen Microbiome in Cattle during Transition from Forage to Concentrate as Well as during and after an Acidotic Challenge. PLoS ONE, 8(12), e83424.

Petri, R. M., Kleefisch, M. T., Metzler-Zebeli, B. U., Zebeli, Q., and F. Klevenhusen. 2018. Changes in the Rumen Epithelial Microbiota of Cattle and Host Gene Expression in Response to Alterations in Dietary Carbohydrate Composition. Appl. Environ. Microb., 84(12), e00384–18.

Petri, R. M., Wetzels, S. U., Qumar, M., Khiaosa-ard, R., and Q. Zebeli. 2019. Adaptive responses in short-chain fatty acid absorption, gene expression, and bacterial community of the bovine rumen epithelium recovered from a continuous or transient high-grain feeding. J. Dairy Sci., 102(6), 5361–5378.

Plaizier, J. C., Khafipour, E., Li, S., Gozho, G. N., and D. O. Krause. 2012. Subacute ruminal acidosis (SARA), endotoxins and health consequences. Anim. Feed Sci. Tech., 172(1-2), 9–21.

Plaizier, J. C., Li, S., Danscher, A. M., Derakshani, H., Andersen, P. H., and E. Khafipour. 2017. Changes in Microbiota in Rumen Digesta and Feces due to a Grain-Based Subacute Ruminal Acidosis (SARA) Challenge. Microb. Ecol., 74(2), 485–495.

Plaizier, J. C., Danesh Mesgaran, M., Derakhshani, H., Golder, H., Khafipour, E., Kleen, J. L., Lean, I., Loor, J., Penner, G., and Q. Zebeli. 2018. Review: Enhancing gastrointestinal health in dairy cows. Animal, 12(s2), s399–s418.

Russell, J. B., and D. B. Dombrowski. 1980. Effect of pH on the efficiency of growth by pure cultures of rumen bacteria in continuous culture. Appl. Environ. Microb., 39(3), 604–610. doi:10.1128/aem.39.3.604-610.1980

Russell, J. B. and J. L. Rychlik. 2001. Factors That Alter Rumen Microbial Ecology. Science, 292(5519), 1119–1122.

Russell, J. B., and D. B. Wilson. 1996. Why Are Ruminal Cellulolytic Bacteria Unable to Digest Cellulose at Low pH? J. Dairy Sci., 79(8), 1503–1509.

Saleem, F., Ametaj, B. N., Bouatra, S., Mandal, R., Zebeli, Q., Dunn, S. M., and D. S. Wishart. 2012. A metabolomics approach to uncover the effects of grain diets on rumen health in dairy cows. J. Dairy Sci., 95(11), 6606–6623.

Staples, C. R., and D. S. Lough. 1989. Efficacy of supplemental dietary neutralizing agents for lactating dairy cows. A review. Anim. Feed Sci. Tech., 23(4), 277–303.

Steele, M. A., Penner, G. B., Chaucheyras-Durand, F., and L. L. Guan. 2016. Development and physiology of the rumen and the lower gut: Targets for improving gut health. J. Dairy Sci., 99(6), 4955–4966.

Turner, J. R. 2009. Intestinal mucosal barrier function in health and disease. Nat. Rev. Immuno., 9(11), 799–809.

Wetzels, S. U., Mann, E., Pourazad, P., Qumar, M., Pinior, B., Metzler-Zebeli, B. U., and Q. Zebeli. 2017. Epimural bacterial community structure in the rumen of Holstein cows with different responses to a long-term subacute ruminal acidosis diet challenge. J. Dairy Sci., 100(3), 1829–1844.

Zebeli, Q., Dunn, S. M., and B. N. Ametaj. 2011. Perturbations of plasma metabolites correlated with the rise of rumen endotoxin in dairy cows fed diets rich in easily degradable carbohydrates. J. Dairy Sci., 94(5), 2374–2382.

Zebeli, Q., Aschenbach, J. R., Tafaj, M., Boguhn, J., Ametaj, B. N., and W.Drochner. 2012. Invited review: Role of physically effective fiber and estimation of dietary fiber adequacy in high-producing dairy cattle. J. Dairy Sci., 95(3), 1041–1056.

Zebeli, Q., and B. U. Metzler-Zebeli. 2012. Interplay between rumen digestive disorders and diet-induced inflammation in dairy cattle. Res. Vet. Sci., 93(3), 1099–1108.

Zebeli, Q., Ghareeb, K., Humer, E., Metzler-Zebeli, B. U., and U. Besenfelder. 2015. Nutrition, rumen health and inflammation in the transition period and their role on overall health and fertility in dairy cows. Res. Vet. Sci., 103, 126–136.

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Abstract

We may be in the midst of a paradigm shift in regard to the role that animal-origin products play in human health. Despite the fact that animal products are amongst the most nutrient-dense foods available to humans, perceptions regarding their effects on health (e.g., increased risks for cardiovascular disease and cancer for dairy and meat, respectively) have become progressively negative. This shift has resulted in important changes in policy and dietary guidelines in several countries, leading to altered patterns of consumption of animal products, and has manifested in trends toward reduced intake of full-fat dairy and beef. Importantly, a substantial body of literature has emerged in recent years that challenges the negative perception linking saturated fats and cardiovascular disease. Furthermore, several recent studies suggest that full-fat dairy consumption may actually reduce the risk of obesity and associated chronic diseases. Similarly, the presumed association between some types of cancer and the consumption of processed or un-processed meat has also been recently put into question. As new studies and more rigorous methods are being used to analyze epidemiological data, we find that strongly held paradigms become weak, as does the degree of confidence on the dietary recommendations to limit these animal products. Further, the constant change in messages received by consumers has led to confusion as to what constitutes healthy dietary habits and may have eroded the confidence in dietary advise arising from nutrition science. Considering the impossibility of using epidemiological research to establish causation, it seems critical to emphasize the importance of randomized control research in order to separate associations from real causal factors. This review has two main objectives: first, to present the historical milestones that have led to the negative shift in consumer's perceptions towards animal products; second, to summarize current dietary recommendations in light of the best scientific evidence available.

Résumé

Nous sommes peut-être en plein changement de paradigme en ce qui concerne le rôle que les produits d'origine animale jouent dans la santé humaine. Bien que les produits d'origine animale comptent parmi les aliments les plus nutritifs disponibles pour l'homme, les perceptions concernant leurs effets sur la santé (par exemple, risques accrus de maladies cardiovasculaires et de cancer pour les produits laitiers et la viande, respectivement) sont devenues progressivement négatives. Cette évolution a entraîné des changements importants dans les politiques et les directives alimentaires de plusieurs pays, ce qui a modifié les habitudes de consommation de produits animaux et s'est traduit par des tendances à la réduction de la consommation de produits laitiers et de viande bovine riches en matières grasses. Il est important de noter qu'un nombre important d'ouvrages ont été publiés ces dernières années qui remettent en question la perception négative du lien entre les graisses saturées et les maladies cardiovasculaires. En outre, plusieurs études récentes suggèrent que la consommation de produits laitiers entiers peut en fait réduire le risque d'obésité et de maladies chroniques associées. De même, l'association présumée entre certains types de cancer et la consommation de viande transformée ou non transformée a également été remise en question récemment. À mesure que de nouvelles études et des méthodes plus rigoureuses sont utilisées pour analyser les données épidémiologiques, nous constatons que les paradigmes fermement ancrés faiblissent, tout comme le degré de confiance dans les recommandations alimentaires visant à limiter ces produits animaux. En outre, l'évolution constante des messages reçus par les consommateurs a entraîné une confusion quant à ce qui constitue des habitudes alimentaires saines et peut avoir érodé la confiance dans les conseils diététiques découlant de la science de la nutrition. Compte tenu de l'impossibilité d'utiliser la recherche épidémiologique pour établir la causalité, il semble essentiel de souligner l'importance des essais contrôlés randomisés afin de séparer les perceptions des facteurs de causalité réels. Cette étude a deux objectifs principaux : premièrement, présenter les étapes historiques qui ont conduit au changement négatif de la perception des consommateurs à l'égard des produits animaux; deuxièmement, résumer les recommandations alimentaires actuelles à la lumière des meilleures preuves scientifiques disponibles.

Importance of animal origin foods and trends of consumption

Given their high nutrient density, animal-origin foods have been staples of the human diet along our evolutionary history. Indeed, evidence of meat and bone marrow consumption in hominins can be traced back to around 2.5-2.8 million years ago (De Heinzelin et al., 1999, Thompson et al., 2019). On the other hand, milk from ruminants was introduced into our diets more recently, at least 8500 years ago (Curry, 2013). Key to early development of mammals, milk constitutes a major source of energy, high-quality protein, and vitamins and minerals such as vitamin D, calcium, and potassium (Smilowitz et al., 2005; Gaucheron, 2005). These nutrients are of particular importance in populations of children experiencing undernutrition, for which this can translate into long-term effects including reduced cognitive and physical development (Black et al., 2013). Data form the WHO shows that stunting (low height for age from chronic under nourishment), affected around 21.3% children under age five in 2019 (Data World Bank, 2020). Importantly, stunting begins in utero and is greatest for the first 1000 days of life, making the adequate nutrition of the mother a key factor (Victora et al., 2010, de Onis and Branca, 2016).

Indeed, beyond its key role in child nutrition, milk and milk-derived products are also major sources of nutrients for adults. The ability of humans to carry on drinking milk through adulthood has developed gradually over the past eight millennia, in association with agriculture and dairying (Curry., 2013), and it is due to the persistence of the enzyme lactase past early childhood. The rise of distinct genetic mutations for lactase persistence developed in at least four different geographic regions on the planet (i.e., lactase hotspots), and, arguably, provided a major selective advantage (Bersaglieri, et al., 2004). Today, one-third of humans produce lactase during adulthood, with prevalence being greater in places like northern Europe, where over 90% of people can drink milk, but also in West Africa, the middle east, and south Asia (Curry, 2013; Liebert et al., 2017).

As the human population continues to grow, demand for animal-origin food follows, and it is estimated that by 2050 the greatest food security challenge will not be the provision of adequate calories, but rather the access to nutrient-dense foods, such as meat and dairy (Nelson et al., 2018). Despite the arguably obvious benefits that animal-origin products can provide to human populations as a source of key nutrients, the image of milk and meat has blemished and their place as components of a healthy diet has been put into question. Indeed, over the past five decades, changes in consumer perception, dietary guidelines, public health messages, and policy, have all resulted in a shift in the patterns of consumption of meat and dairy products, particularly those of higher fat content.

A generalized "fear of fats" has spread in recent decades and shaped consumer's choices, who nowadays tend to look for reduced-fat foods as healthier alternatives to full-fat options, in hopes of reducing intake of fat and 'calories', and the risk of heart problems and obesity. Simultaneously, milk avoidance has become more prevalent, with a linear decrease in Canada, starting at the end of the 1970's (Figure 1; Statistics Canada), and new beverages of plant origin with low contents of saturated fats have become available, replacing cow's milk in the diet (e.g., the so –called soy and almond milks). Furthermore, the most recent nutrition guidelines in Canada suggest, to limit our intake of foods containing saturated fats, which is based on the interest in reducing LDL-cholesterol (Health Canada, 2019). In contrast to current perceptions and official dietary advice, an important body of literature has emerged over the past decade that challenges the contemporary views associating saturated animal fat consumption to human disease. Such studies suggest that dairy in general, as well as full-fat dairy, may decrease the risk for CVD. Furthermore, recent studies suggest that full fat dairy may actually protect from obesity and associated chronic diseases.
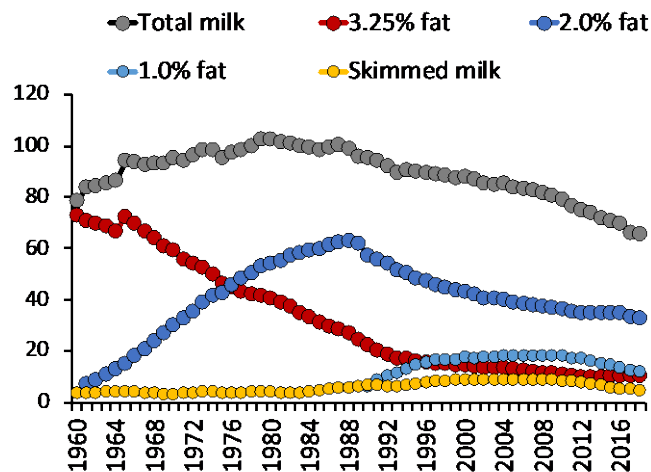
Figure 1. Consumption trends of fluid milk in Canada from 1960 to 2018
Source: Statistics Canada

A Note on the Assessment of Evidence: *An example from the association between meat and cancer*

In 2015, the world health organization (WHO) released a report where a meta-analysis of 10 cohort studies yielded a positive relationship between red and processed meat and colorectal cancer (Bouvard et al., 2015). The WHO initially considered 800 observational studies regarding the association between cancer and meat. However, their findings are based on 56 studies looking at colorectal cancer. Their assessment estimated a 17% increased risk per 100 g/d of red meat, and a 18% increase per 50 g/d of processed meat. This resulted in the classification of red meat as ''probably carcinogenic'' and processed red meat as ''carcinogenic''. Importantly, these conclusions are primarily based on observational studies which are limited in their ability to establish causal inferences and are at high risk of confounding.

In contrast, Jonhston et al. (2019) performed 4 parallel systematic reviews studying the impact of red and processed meat on cardiometabolic and cancer outcomes suing both randomized trials and observational studies. A panel of 14 members from 7 countries was formed to provide a recommendation based on the quality of the evidence. The panel recommended that adults continue the consumption of both types of meat. Importantly the recommendations from this committee were based on strict methodology, including the Nutritional Recommendation (NutriRECS) guideline development process and GRADE (Grading of Recommendations, Assessment, Development and Evaluation) methods (Johnston et al., 2019).

To explain this discrepancy, an analysis by Fogelholm et al. (2015) shows how epidemiological research on meat and chronic disease can be riddled with confounding factors. For instance, there is a strong association between meat consumption and a 'lower-quality' diet, smoking, and lack of physical activity in both men and women, which complicates interpretation of observational studies (Fogelholm et al., 2015). Furthermore, evidence for claims related to food and human

health come from a range of studies with varying degrees of causal strength, going from observational studies to controlled randomized clinical trials. Young and Karr (2011) give some perspective on the frequency at which observational claims fail to replicate, at an alarming rate of 80%. Further, in about 10% of the cases, when the claims from observational studies were tested in clinical trials they moved significantly in the opposite direction (Young and Karr, 2011). These authors suggested that ''any claim coming from an observational study is most likely to be wrong – wrong in the sense that it will not replicate if tested rigorously''. Therefore, assessing the observational evidence that relates dietary intakes to common disease outcomes is, at the very least, problematic (Prentice, 2014). For instance, the validity of observational studies can be compromised by the necessary reliance placed on self-reported food intakes and can further be complicated by the effects of confounders, such as lifestyle factors that may impact the association. This highlights some of the problems with claims originated from ecological studies, such as the diet-heart hypothesis, which now seems to lose validity in the face of stronger pieces of evidence, such as RCTs and prospective cohort studies, which contradict its original presuppositions (e.g., Micha and Mozaffarian, 2008; Ramsden et al., 2016). While bearing these limitations in mind, the interpretation of observational studies relating dairy intake and health outcomes can be in many instances a useful starting point for investigation and validation. Regardless, causation may only be derived from properly controlled experimentation.

The Diet-heart Hypothesis - Background and Classic Studies

The now prevailing concept that saturated fats of animal origin are detrimental to human health can be traced to some suggestive pieces of evidence originated in the 20th century. First, the ground-breaking studies of Ignatowski in 1908 and Anichkov in 1913 demonstrated the ability of animal fats and, specifically, cholesterol, to cause atherosclerotic lesions, raise plasma cholesterol, and cause death in rabbit models of atherosclerosis (Konstantinov et al., 2006). Second, during the 1950's, Ancel Keys produced epidemiological data that seemed to identify dietary fat as a major cause of heart disease. In two commonly known studies, Keys and collaborators showed some seemingly strong associations between national death rates for middle-aged men from arteriosclerotic and degenerative heart disease and the proportion of fat-calories available in their national diets (Keys, 1953; Keys et al., 1966). His data relating availability of energy from fat and cardiovascular-related death led him to conclude that "dietary fat somehow is associated with cardiac disease mortality, at least in middle age". These studies propelled the extensively known diet-heart hypothesis that related dietary factors to the incidence of cardiovascular disorders. The validity of this hypothesis was quickly challenged by Yerushalmy and Hilleboe (1957), and its acceptance has remained far from unanimous ever since it was first proposed. In their methodological note, the authors attempted to evaluate whether the proposed hypothesis could actually reflect "known or ascertainable facts" which may allow for the generalization of this premise. The authors pointed at several limitations, including 1) small sample size (i.e., only 6 countries were used in Key's original study); 2) the possible effects of unaccounted confounders (e.g., underreporting in countries with lower economic status who cannot afford meat and dairy); 3) the fact that no actual data on fat consumption was used (i.e., availability was used); 4) the lack of specificity of the relationship (i.e., protein consumption also related to death); 5) variation in deaths from cardiac disease are largely variable across countries at any given fat availability category. Some interesting points emerging from their methodological analysis include: first, the

strength of the association is greatly reduced when more countries are included (n= 22); second, the relationship is not specific to fat; third, "almost no association" was found when correlating fat or protein with all causes of death. This last point merits attention because CVD is the leading cause of death in industrial countries, and consequently, the relationship in Keys' studies would be expected to also hold for all-cause mortality. More recently, others (Willet, 2012) have pointed out that the countries chosen by Keys to represent low fat intake and low incidence of CVD were in fact less industrialized and showed differences in smoking habits, physical activity and obesity, thus complicating the generalization of the diet-heart hypothesis.

Dietary Lipids and CVD Risk

The importance of cholesterol in the development of CVD in humans was first suggested by data extracted from the Framingham study, which enrolled 5127 men and women aged 30–59 years in Massachusetts, starting between 1948 and 1950. Following six years of longitudinal evaluation, cholesterol was identified as one of three risk factors for CVD (Kannel et al., 1961)[1]. Several other studies between the 1950's and 1980's found positive associations between serum (total) cholesterol and risk of CVD. With the advent of techniques to identify and measure circulating lipoproteins, it was further shown that cholesterol contained in very low-density lipoproteins (**VLDL-C**), as well as low-density lipoproteins (**LDL-C**; i.e., 'bad cholesterol'), correlates positively with CVD risk, while that found in high density lipoproteins (**HDL-C**; i.e., 'good cholesterol'), correlated negatively (see review by Parodi, 2009). In fact, LDL-C became the lead marker for atherogenicity and CVD risk, and thus, the main target and factor guiding CVD treatments in the last few decades (Stone et al., 2014). However, the role of LDL-C recently has been put into question as it is considered a very poor predictor of CVD (Sachdeva et al., 2009; Ravnskov et al., 2018). On the other hand, HDL-C (an indicator of cholesterol efflux) levels are used as a marker of reduced CVD risk (Rohatgi et al., 2014; Monette et al., 2016), particularly when used as a ratio of total cholesterol to HDL-C (Castelli, 1988). In addition, increased triglyceride to HDL-C ratio is a more powerful predictor of coronary heart disease (Luz et al., 2008).

While much of the focus has been historically placed on cholesterol, other factors seem to be important to predict CVD risk. Some of these include obesity, serum triglycerides, inactivity, hypertension, cigarette smoking and diabetes. Results from the Framingham cohort illustrate the importance of these cofactors, as, for example, accounting for glucose intolerance, high systolic blood pressure, smoking, and left ventricular hypertrophy increased CVD risk to 60.2, compared to only 3.9 when cholesterol alone was used (Kannel et al., 1979). Furthermore hyperinsulinemia has been identified as the most important predictor of CVD in some studies, (Després et al., 1996, García et al., 2011), which may explain the association between CVD and previously identified risk factors such as obesity.

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[1] Indeed, interest in cholesterol seemed justified, as atheromatous lesions are rich in free and esterified cholesterol, relative to normal arterial walls (Windaus, 1910). Moreover, cholesterol alone can cause atheromatous lesions in the vascular wall (Anitschkow and Chalatow, 1913).

Saturated Fats and Blood Lipids

The ability of saturated fatty acids (**SFA**) to raise blood LDL-C is consistent across the literature, and it is well documented (Micha and Mozaffarian, 2008). Interest in this relationship stems from hypothesis that LDL particles may increase cholesterol accumulation in arterial walls, facilitating the formation of atheromatous plaque, and therefore increasing CVD risk (Kruth et al., 2001). Consequently, efforts have been made to determine the atherogenic potential of individual SFA as a marker of CVD. For example, Ulbricht and Southgate (1991) proposed the atherogenic index, which is calculated by dividing the sum the SFA lauric (12:0), myristic (14:0) and palmitic (16:0), by the sum of omega 3 and 6, 18:1c9, and other monounsaturated fatty acids. Based on Hegsted's work (1965), each factor is multiplied by an empirical constant according to its capacity to raise or decrease cholesterol, using a value of 1 for all fatty acids and a value of 4 for 14:0. Because these presumably atherogenic fatty acids (12:0, 14:0, 16:0) represent 30-40% of cow's milk triglycerides (Jensen, 2002; O'Donnell-Megaro et al., 2011), some have concluded that dairy fat is a potential cholesterol-raising food, and, consequently, consumers have reduced the consumption of full-fat dairy (Wang and Li, 2008). Simultaneously, official advice has focused on reducing the consumption of fat and saturated fat specifically. The dietary fat guidelines introduced in 1977 (US) and 1983 (UK) (Harcombe et al., 2015) recommend to 1) reduce overall fat consumption to 30% of total energy intake and 2) reduce saturated fat consumption to 10% of total energy intake. It is important to note that, with these guidelines, the ability of SFA to raise blood HDL-C, and to reduce CVD risk, is implicitly ignored (Parodi, 2009). In fact, a meta-analysis of 60 controlled trials showed that SFA have no effect on the ratio of total cholesterol to HDL-C (lower is better) when SFA replace dietary carbohydrates (Mensink, et al., 2003). Furthermore, the allegedly "atherogenic" lauric acid reduces the ratio, mostly by increasing HDL-C. These observations challenge the notion that SFA are indeed atherogenic and that SFA sources like milk fat may have health-adverse effects.

Furthermore, low fat diets increased circulating triglycerides and the ratio of total cholesterol to HDL-C (**Total:HDL-C**; i.e., the atherogenic ratio), both proxies for increased CVD risk. In this way, elevations observed by reducing dairy fat intake cast some concerns about the efficacy of low-fat diets to reduce CVD risk. Arguably, increased CVD risk may actually result from following this type of low-fat approach. Although factors like adiposity and insulin resistance of subjects may have influenced the responses to low-fat diets in this study, it is evident that the effects of such diets on the commonly used biomarkers (e.g., LDL-C, HDL-C, Total:HDL-C, and triglycerides) were certainly not what many may expect in terms of alleviation of CVD risk. Moreover, the validity of CVD biomarkers like LDL-C has dwindled progressively, and the role of lipoproteins on CVD has shifted focus into their size (i.e., small and dense LDL profile is worse), number (i.e. Apolipoprotein B as a marker of atherogenic particles), and oxidation propensity, rather than their cholesterol content (Lamarche et al., 1997; Krauss, 2005; Parodi, 2009). An example comes from the guidelines of the American Heart Association, who, based on a recent review of available data, reported being unable to find evidence to support continued use of specific LDL–C and/or non-HDL–C treatment targets. In this way, the "bad cholesterol" is no longer the main factor guiding treatment (Stone et al., 2014).

Even leaving CVD lipid biomarkers aside, under the diet-heart hypothesis, the effects of SFA on actual clinical outcomes should reflect harmful consequences on cardiovascular endpoints.

Contrary to this expectation, a meta-analysis of prospective cohort studies that followed 347,747 subjects for 5 to 23 years found no association between saturated fat intake and CVD, both fatal and non-fatal (Siri-Tarino et al., 2010). Similarly, in a systematic review and meta-analysis of prospective cohort studies and RCTs (n = 78) with 649,812 participants, Chowdhury et al. (2014) reported no increase of relative risk of coronary outcomes associated with dietary or circulating SFA. Moreover, the authors reported an inverse association between circulating margaric acid (17:0, a marker of dairy fat intake) and coronary disease. Taken together, available evidence from prospective epidemiologic studies and RCT does not support guidelines encouraging reduced saturated fat consumption, particularly those from dairy. Whether this evidence can be considered sufficient to totally vindicate SFA and dairy, is still a matter for discussion; however, currently available data suggest the heavy focus on saturated fats may be not only unnecessary, but perhaps also detrimental. This is particularly true when considering that dairy fats may have been replaced with industrial *trans* fats of plant origin (e.g. margarines with high *trans* fat content), as well as refined sugars (i.e., fructose). Some trends exemplifying these dietary substitutions can be seen in Figure 1. Full fat milk has been partially substituted with lower-fat versions, while soft drinks consumption and fructose has risen in linearly. Importantly, there are cogent reasons to believe that the simultaneous reduction in consumption of some dairy products and the increase industrial *trans* fats and sugars could be detrimental. For example, non-ruminant (i.e., industrial) *trans* fats are nowadays recognized as harmful (Micha and Mozaffarian, 2008) and they relate strongly to heart disease and all-cause mortality (Oomen et al., 2001; de Souza et al., 2015). Similarly, a growing body of evidence indicates that most US adults currently consume excess added sugar (a source of fructose), and this is significantly associated with obesity, metabolic syndrome, and CVD mortality (Johnson et al., 2009; Lustig et al., 2010; Yang et al., 2014). In fact, the American Heart Association has recommended the reduction of dietary sugar intake by more than half (Johnson et al., 2009).

Saturated Fats and CVD Risk and CVD Mortality

As discussed previously, the relationship between SFA consumption and CVD is not straightforward, and it was historically derived from the diet-heart hypothesis, with two important premises: 1) SFA can influence circulating cholesterol (i.e., increase LDL-C), and 2) cholesterol is a risk factor for CVD. The resulting assumption was, therefore, that SFA consumption can cause CVD. Given the disconnect between SFA consumption and the anticipated clinical CVD outcomes (e.g., Siri Tarino et al., 2010; Chowdhury et al., 2014), the strength of the diet-heart hypothesis has been questioned. Considering that dairy products may have protective effects against obesity, T2D, and metabolic syndrome, it is important to elucidate whether this may also be true for CVD, which remains a major cause of death in the United States (Mozaffarian et al., 2015). When looking at the effects of dairy consumption on CVD risk factors, the most salient finding is that, contrary to expectations, reducing SFA intake from dairy increases CVD risk, as determined by commonly used markers (Lefevre et al., 2005). This sobering observation seems to receive further support from other studies that report significant associations between milk-derived fatty acids and a more favorable LDL particle size distribution (i.e., reduction in small dense LDL particles; Sjogren et al., 2004). Furthermore, some SFA found in milk fat, such as lauric acid, are actually associated with a reduction of CVD risk (Micha and Mozaffarian., 2010). Importantly, these pieces of

evidence align with the solid, general observation, that SFA are neutral or even beneficial in terms of CVD risk.

In contrast, a recent meta-analysis by the presidential advisory of the AHA concluded that saturated fatty acids (SFA) should be replaced with unsaturated fats, in particular with PUFA (Sacks et al., 2017). Using data from randomized controlled trials, Sacks et al., (2017) showed that lowering intake of SFA by replacement with PUFA results in 29% lower CHD. This is in contrast to the meta-analysis of Hamley (2017), who reported replacing saturated fat with PUFA had no effect on major CHD events or CHD mortality. A beneficial effect of PUFA was only observed when _inadequately_ controlled trials were included. Those trials were deemed inadequately controlled when important factors, other than the SFA replacement by PUFA, were different between groups (Hamley, 2017). In support of this, in an extensive review of the meta-analyses of observational and controlled studies looking at the replacement of SFA with PUFA, Heileson (2019) points out that 3 of the 4 core clinical trials used in the analysis of Sacks et al., (2017) contained design and methodological flaws, which biased their conclusions. Briefly, the Finnish Mental hospital study [FMHS; (Turpeinen et al., 1979)], the Oslo Diet-Heart Study [ODHS; (Leren, 1970)], The Los Angeles Veterans Administration Trial [LA Vets; (Dayton and Pearce, 1969)], The British Medical Research Council study [MRC; (MRC, 1968)], were included in the AHA study by Sachs et al. (2017) despite several flaws including differences in smoking habits between groups, confounding dietary factors, lack of individual randomization and low adherence across 3 of them (ODHS, LA Vets, and FMHS). In view of the results of all meta-analyses included in his review, Heileson (2019) concludes that observational and properly controlled clinical trials show lack of association of SFA and heart diseases, suggesting the current stance of the AHA may need to be revaluated.

The Effects of Dairy on CVD

Focusing on evidence from prospective data, Elwood et al., (2008) conducted a meta-analysis of 15 prospective cohort studies reporting the association between milk and dairy consumption and the incidence of vascular diseases in the UK. The relative risk (RR) of stroke and/or heart disease was significantly reduced in subjects with high milk or dairy consumption (RR =0.84 and 0.79, respectively), compared with the risk in those with low consumption. These findings highlight once more, the disconnect between the hypothesized effects of SFA-containing foods like dairy, and the actual clinical outcomes of interest. Similarly, a systematic review of the available literature indicates that most studies do not support the expected effects of dairy fat on CVD, and that discrepancies may be associated to country-specific effects (Kratz et al., 2012). Specifically, the Nurses' Health study (from the US) found a consistent positive association between dairy fat intake and CVD, while 11 other studies across Europe, Costa Rica, and Australia, showed either no association or an inverse relationship between CVD and dairy fat intake. Only one of these 11 studies reported a discrepancy, as it found an inverse association in men, but a positive one in women (Kratz et al., 2012). The authors suggested that residual confounding from lifestyle factors associated with dairy intake, as well as differences in food sources of dairy fat, may help explain the discrepancy between US and non-US data. Relevant to this point, the recently published results from the Prospective Urban Rural Epidemiology (**PURE**) study evaluated the effects of dairy consumption on death and major CVD events across 21 countries and 5 continents in an 9-year

follow-up (Dehghan et al., 2018). Dietary intakes of dairy products for 136,384 individuals were recorded using country-specific validated food frequency questionnaires. Dairy foods evaluated included milk, yoghurt, and cheese, and these were grouped into whole-fat and low-fat dairy. Dairy intake above 2 servings per day reduced the risk of total mortality, CVD mortality, major CVD, and stroke, relative to no intake. Similarly, whole-fat dairy (> 2 servings per day) was inversely associated with total mortality and major CVD. Interestingly, the CVD response to whole-fat dairy appeared to be dose-responsive, as it increased progressively from <0.5 to 0.5-1, 1-2, and >2 servings per day. Cheese consumption (>1 serving per day) was associated with reduced mortality and major CVD, while the effect of butter was neutral (i.e., no increase in risk). The PURE study suggests that dairy intake, especially whole-fat dairy, might be beneficial for preventing deaths and major cardiovascular diseases. Moreover, there seems to be no disadvantage associated with the consumption of full-fat dairy, compared with the low-fat counterparts. The authors conclude that consumption of dairy products should not be discouraged and perhaps should even be encouraged, particularly in low-income and middle-income countries where dairy consumption is low.

The Effects of Dairy on other health outcomes

Obesity and Type 2 Diabetes

One arguably important reason for the current trends of dairy fat avoidance (Figure 1) is related to the interest in reducing excess energy intake. The common presumption is that dairy fat can be stored as body fat and thus contribute to weight gain, obesity, and cardiometabolic risk. This has driven dietary guidelines to recommend the consumption of low-fat dairy (Jensen et al., 2014). In contrast to guidelines and prevailing public sentiment, available evidence indicates dairy fat consumption is not related with the risk of weight gain. The comprehensive review of Kratz et al. (2013), which used a combination of observational and controlled studies, indicates that dairy fat consumption, both recorded or assessed via odd-chain fatty acid content in blood (e.g., 15:0 and 17:0), was inversely related with obesity risk. Similar findings were reported in a cross-sectional evaluation of full-fat milk consumption in three-year-old children (Beck et al., 2017). The multivariate analysis included potential demographic and nutritional confounders. The authors reported reduced odds for severe obesity in association with higher milk fat consumption, suggesting a protective effect of dairy fat against obesity in three-year-olds. Similarly, in a prospective cohort study of 18,438 healthy middle-aged women followed during 11 years and belonging to the Women's Health Study, greater consumption of total dairy products reduced the risk of becoming overweight or obese. Furthermore, the lowest risk was observed at the highest quintile of high-fat dairy product intake (Rautiainen et al., 2016). Finally, in a meta-analysis of 29 RCTs, Chen et al. (2012) reported dairy consumption does not increase body weight gain or body fat gain. Moreover, dairy consumption results in modest beneficial effects on weight loss in short-term and energy-restricted RCTs.

Americans today are eating 25-28% more calories per day, which amounts to an extra 425-800 kcal/d relative to 1961, and is explained mostly by an increase in intake of cereal grains and vegetable oils (Guyenet and Schwartz, 2012). Poortvliet et al. (2007) reported an increase in voluntary energy intake in subjects fed a higher carbohydrate (Fetuccine-based meal) relative to a higher protein (Chicken-based) meal. This was observed despite meals being isoenergetic and was

explained by reduced hunger throughout the day when the chicken-based meal was offered (Poortvliet et al., 2007). These observations illustrate an important effect of diet on dysregulation of intake (Guyenet and Schwartz, 2012), which recently has been shown to also be impacted by the consumption of processed foods (Hall et al., 2019). In a randomized controlled trial, Hall et al., (2019) compared two iso energetic diets varying in degree of processing. Relative to a diet rich in dairy, meat, fruits and vegetables, an ultra-processed diet resulted in higher energy intake, and increased body weight (+1.8 kg) and fat mass (0.7kg) after 14 d of intervention (Hall et al., 2019).

Type 2 diabetes (**T2D**) is rapidly rising worldwide, paralleling the epidemic increase in obesity. Because of its high content of calcium, magnesium, vitamin D, and whey proteins, which could reduce insulin resistance, dairy products could be hypothesized to protect against T2D (Rice et al., 2011). The meta-analysis of Aune et al. (2013) evaluated the association between intake of dairy products and the risk of T2D from prospective cohort and nested case-control studies (n=17). Non-linear, inverse associations were found between the risk of T2D and intakes of dairy products, low-fat dairy, yogurt, and cheese, the latter being the highest in fat content. The risk responses to dairy intake were dose-dependent, and flattened at higher intakes. Interestingly, high fat dairy did not alter the T2D risk in this study, although a meta-analysis focused specifically on butter consumption (Pimpin et al., 2016; 11 country-specific cohorts and 201,628 participants) reported butter intake was associated with a reduction of T2D risk. This discrepancy may suggest that the effects of dairy fat may be food-specific (e.g. cheese different from butter), a concept that merits further investigation. Other recent meta-analyses add support to the protective effects of dairy consumption against T2D (Forouhi et al., 2014; de Souza et al., 2015; Yakoob et al., 2016). For example, the prospective associations between circulating fatty acids in phospholipids and T2D were reported in individuals from the EPIC-InterAct case-cohort study (17,928 T2D subjects and 16,835 participants in a random subcohort; Forouhi et al., 2014). By design, this study combines the temporal sequence and power advantages of a larger prospective cohort, with the measurement efficiency of a case-control. Forouhi et al., 2014 reported reduced hazard ratios for incident T2D in association with the odd chain SFA 15:0 and 17:0, both of which are mostly derived from dairy products. Similarly, using two prospective cohorts with 3333 adults aged 30 to 75 years, free of T2D at baseline, and followed during 15 years, Yakoob et al. (2016) found that individuals at the highest quartile of plasma 15:0, 17:0, and *t*-16:1n-7 had reduced risk of incident diabetes mellitus (-44%, -43%, and -53%, respectively; Figure 2). This last finding is of particular interest given that other studies have showed that circulating *trans*-palmitoleic acid (*t*-16:1n-7) is associated with lower insulin resistance, atherogenic dyslipidemia, and incident diabetes (Mozaffarian et al., 2010; de Souza et al., 2015). Importantly, whole-fat dairy consumption is most associated with elevated plasma concentrations of *trans*-palmitoleic acid (Mozaffarian et al., 2010). Whether the apparently beneficial effects of dairy on T2D risk are mediated by *trans*-palmitoleic acid or other components of dairy, remains to be experimentally elucidated; regardless, this possibility constitutes an exciting new direction for fatty acid research.
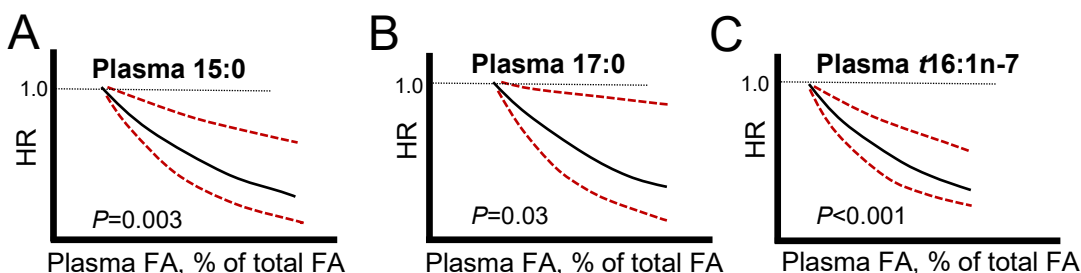
Figure 2. Circulating biomarkers of dairy fat intake and risk of incident diabetes mellitus in two large prospective cohorts using 3,333 adults in a 15-year follow-up (Adapted from Yakoob et al., 2016). Solid-black and dashed-red lines represent hazard ratios (HR) and their 95% confidence intervals, respectively, for plasma A)15:0, B)17:0, and C) *t*-16:1n-7.

Metabolic Syndrome

Metabolic syndrome (**MetS**) consists of a cluster of cardiovascular risk factors that include central obesity, hyperglycemia, hypertriglyceridemia, low HDL-C, and hypertension (Alberti et al., 2009). Moreover, MetS is closely associated with CVD risk, T2D, all-cause mortality and cancer (Saely et al., 2007; Wu et al., 2010; Esposito et al., 2012). Indeed, epidemiological evidence suggests that several types of cancer such as liver, gallbladder, and pancreas cancer are related to obesity, which could be mediated by insulin resistance (Calle and Kaaks, 2004).

Despite the recognition of the potential of dairy products to prevent or alleviate CVD, T2D and blood pressure in adults (e.g., 2010 Dietary guidelines for Americans; USDA/USDHHS, 2010), data on the relationship between dairy consumption and MetS is very limited. Chen et al. (2015) evaluated currently available data from cross-sectional/case control studies (n=16) and prospective cohort studies (n=7) in two different meta-analyses. Comparing high vs. low dairy products intake, both meta-analyses showed a reduction in MetS risk with high dairy consumption, an observation that was maintained when the studies were evaluated by stratified subgroups (e.g. geographic region, sex, type of dairy, and follow-up duration). Finally, a dose response analysis of prospective cohorts in the same study showed an inverse relation between MetS risk and dairy consumption (Chen et al., 2015). Interestingly, the reduction in risk became evident when dairy intake was higher than 2 servings per day, and behaved linearly thereafter. This would suggest a minimum amount of dairy may be needed to positively impact MetS risk in a significant manner.

Cancer incidence

The link between dairy and cancer has been the subject of multiple studies in recent years, and it is an active area of discussion. A recent overview analysis of published meta-analyses and systematic reviews aggregated all results looking at the relationship between dairy consumption and cancer incidence (Jeyaram et al., 2018). Their analysis concluded that available studies were mostly low to moderate in terms of quality, which limits the capacity to evaluate this association and provide guidelines for consumption dairy.

Prostate cancer: Plaza et al. (2019) summarized the available reviews and meta-analyses investigating the link between dairy consumption and prostate cancer. Their analysis shows that although some data support a positive association between dairy and prostate cancer, there is a high degree of variability in the quality of published studies, including weak control of confounding factors, rendering this association inconclusive (López-Plaza et al., 2019).

Bladder cancer: A meta-analysis of cohort and case-control studies found high milk intake was associated with decreased risk of bladder cancer (Mao et al., 2011). In contrast, a recent meta-analysis of similar studies by Bermejo et al. (2019) reported that high intake of whole milk increased bladder cancer risk, whereas medium intake reduced risk, although authors warned results may be affected by high heterogeneity of the studies included in the analysis.

Colorectal cancer: Barrubés et al. (2019) showed cheese consumption was inversely related to colorectal cancer in a meta-analysis of 29 observational studies, however other types of dairy such as fluid milk and fermented milk were not associated to colorectal cancer. A randomized controlled trial, published in the Journal of the American Medical Association, showed dairy intake reduces proliferative activity of colonic epithelial cells and restores markers of normal cellular differentiation in patients determined to be at risk of colonic neoplasia (Holt et al., 1998). Such observation could be explained by calcium and/or vitamin D in milk (Holt, 1999) similar to studies in rats where markers of dimethylhydrazine-induced colorectal cancer were reduced by dietary calcium (Pierre et al., 2008). Furthermore, a meta-analysis of 60 observational studies and 26,335 cases of colorectal cancer, showed that dietary calcium and dairy intake were inversely related to cancer risk (Huncharek et al., 2008).

Breast cancer: Several meta-analyses of prospective cohorts and/or case-control studies have looked at the association between breast cancer and dairy intake, showing either no association (Missmer et al., 2002) or an inverse association (Dong et al., 2011, Zang et al., 2015). For instance, in the largest meta-analysis including 22 prospective cohort and 5 case-control studies across Asian and Western populations, Zang et al. (2015) report effects may depend on dairy type, dose, and time, such that high (>600 g/d) and intermediate dairy consumption (400-600 g/d) reduce breast cancer more pronouncedly, whereas a similar effect by fermented dairy products is detected after 10 years of follow up, but only in American women. The mechanism behind these associations is not yet clear, but may be partly mediated by anti-inflammatory effects of dairy. Rashid et al. (2006) reported fermented milk reduces the growth rate of mammary tumors in a murine model, an effect mediated by reduced production of pro-inflammatory cytokines. In line with this observation, a systematic review of RCTs (Ulven et al., 2019) documented a significant reduction of inflammation in both healthy and unhealthy individuals consuming dairy. Furthermore, the anti-cancer effects may also be related to bioactive components found in cow's milk (Parodi, 2009), some of which are reviewed in the following section.

Milk components with anticancer properties

The mechanistic modes of action by which milk and dairy products may be protective against cancer, CVD and other health outcomes is likely complex and still requires investigation. It is important to bear in mind that bovine milk contains an outstanding number of bioactive

components (Park, 2009), which may interact additively, synergistically, or antagonistically. The heavy focus on single nutrients during the past decades (e.g., saturated fats should be avoided) has proven narrow in scope and of limited ability to predict health outcomes. As proposed by others (Mozaffarian, 2014), food-based guidelines that reduce confusion for consumers and are based on prospective evidence for effects on clinical endpoints are needed.

Bovine milk fat is highly complex, containing up to 400 different types of lipids, and although a high proportion of those are saturated, others, which are considered to be bioactive, are also present in milk fat (Jensen, 2002), and may explain the previously discussed positive health outcomes associated with dairy consumption. Most milk lipids (approx. 98%) are found in the form of triglycerides, with the remainder composed of diglycerides, phospholipids, and cholesterol (Jensen, 2002). The following is a list of some molecules found in milk which have some potential as anticarcinogenic agents:

Butyric acid (4:0): Ruminant milk is the main dietary source of butyric acid as it derives from ruminal fermentation processes. Butyric acid has been shown to inhibit chemically induced mammary tumors in rats (Yanagui et al., 1993; Belobrajdic and McIntosh, 2000). As reviewed by Parodi (2009), butyric acid has anti-inflammatory properties, which may be associated with its potent anticancer effects.

Conjugated linoleic acid (CLA): Bovine milk fat contains over 20 positional and geometric CLA isomers, although *cis*-9, *trans*-11 CLA (also known as rumenic acid) is the predominant isomer and accounts for ~75-90% of the total CLA (Lock and Bauman, 2004). Importantly, these isomers are unique to ruminant-origin products since they are produced in the rumen by microbial biohydrogenation (BH) of dietary PUFA. The main pathway of BH yields *cis*-9, *trans*-11 CLA and other downstream intermediates such as *trans*-9 18:1, and may be progressively shifted by dietary factors impacting ruminal bacteria, leading to increased production of other isomers such as *trans*-10, *cis*-12 CLA, a potent inhibitor of mammary lipogenesis (Bauman and Griinari, 2001, Jenkins et al., 2008, Rico et al., 2015). Importantly, the most studied CLA isomers in humans or in animal models are *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA, given the anticarcinogenic and anti-inflammatory properties of the former and the anti-lipogenic properties of the latter (Parodi, 2009, Foote et al., 2010, Kennedy et al., 2010).

Sphingolipids: they represent 25 - 35% of milk phospholipids (Jensen, 2002) and show potential to reduce the incidence of inflammation-related chronic diseases, by acting directly on the microbiota, on the action of bacterial lipopolysaccharide (LPS), and on anti-inflammatory receptors (Norris and Blesso, 2017). In addition, sphingolipids such as sphingomyelin have been shown to inhibit certain types of cancer in mice (Vesper et al., 1999). Importantly, the sphingolipid content and profile of milk may vary depending on the cow's physiological status (Jensen, 2002), but the effects of cow diet on their concentrations in milk fat are not known.

Branched chain fatty acids (BCFA): they are bioactive food components that make up about 2% of dairy fat in cows (Kuzdzal-Savoie, 1964, Ran-Ressler et al., 2014). The anti-cancer capacity of 15:0-iso, a BCFA, was similar to that of CLA (Wongtangtintharn et al., 2004). In addition, 15:0-iso inhibited the growth in vitro and in vivo of various cancer cell lines by inducing apoptosis without toxic side effects (Yang et al., 2000). Branched chain FA are produced by rumen

microorganisms, for which they constitute an important part of cell membranes. Once synthesized in the rumen, these fatty acids are absorbed in the intestine, delivered to the mammary gland and incorporated into milk fat (Fievez et al., 2012, Baumann et al., 2016). Similar to other milk FA, their concentrations can also be modified by the diet, in particular fiber and PUFA content of diets have been shown to alter the concentrations of these FA in milk (Villeneuve et al., 2013, Saliba et al., 2014, Baumann et al., 2016).

Conclusion

Production of nutrient-dense foods will be a major challenge as global demand from a growing population continues to increase. Re-evaluation of the validity of classic literature and the emerging abundance of new evidence over the past decade, strongly contradict the long-held idea that dietary saturated fats cause adverse effects on health. Moreover, as shown in this review, current evidence indicates that dairy products, including full-fat dairy, may exert protective effects on metabolic health, reducing the incidence of obesity, T2D, MetS, CVD, several types of cancer and all-cause mortality. The mechanisms behind such effects has not yet been completely elucidated, but may be related to the wide range of bioactive compounds, such as butyric acid, conjugated linoleic acid, branched chain fatty acids and sphingolipids found dairy. In light of this, a general call to revise the guidelines on dairy consumption seems strongly justified and necessary, particularly as dairy products may help combat the spread of chronic diseases. Moreover, the historic focus on individual nutrients (e.g., fat, calories) has proven limited in terms of predicting clinical outcomes. In this sense, a whole-food approach to studying the effects of the ensemble of nutrients contained in animal-origin foods on human health outcomes seems warranted. Lastly, policy changes should be guided by a more nuanced interpretation of observational studies, and reflect the higher value of repeatable, randomized controlled studies, as the latter may provide insights on causality and the role of dairy on public health.

References

Alberti, K.G.M.M., Eckel, R.H., Grundy, S.M., Zimmet, P.Z., Cleeman, J.I., Donato, K.A., Fruchart, J.C., James, W.P.T., Loria, C.M. and S.C. Smith Jr. 2009. Harmonizing the metabolic syndrome: a joint interim statement of the international diabetes federation task force on epidemiology and prevention; national heart, lung, and blood institute; American heart association; world heart federation; international atherosclerosis society; and international association for the study of obesity. Circulation 120:1640-1645.

Anitschkow N. und S. Chalatow. 1913. Ueber experimentelle Cholester-insteatose und ihre Bedeutung fuer die Entstehung einiger pathologischer Prozesse. Zentrbl. Allg. Pathol. Pathol. Anat. 24:1–9.

Aune, D., Norat, T., Romundstad, P. and L.J. Vatten. 2013. Dairy products and the risk of type 2 diabetes: a systematic review and dose-response meta-analysis of cohort studies–. Am. J. Clin. Nutr 98:1066-1083.

Barrubés, L., N. Babio, N. Becerra-Tomás, N. Rosique-Esteban, and J. Salas-Salvadó. 2019. Association Between Dairy Product Consumption and Colorectal Cancer Risk in Adults: A Systematic Review and Meta-Analysis of Epidemiologic Studies. Advances in Nutrition 10(suppl_2):S190-S211.

Bauman, D. E. and J. M. Griinari. 2001. Regulation and nutritional manipulation of milk fat: low-fat milk syndrome. Livest. Prod. Sci. 70(1-2):15-29.

Baumann, E., P. Chouinard, Y. Lebeuf, D. Rico, and R. Gervais. 2016. Effect of lipid supplementation on milk odd-and branched-chain fatty acids in dairy cows. J. Dairy Sci. 99(8):6311-6323.

Beck, A.L., Heyman, M., Chao, C. and J. Wojcicki. 2017. Full fat milk consumption protects against severe childhood obesity in Latinos. Prev. Med. Rep. 8:1-5.

Belobrajdic, D. P. and McIntosh, G. H. 2000 Dietary butyrate inhibits NMU-induced mammary cancer in rats. Nutrition and Cancer, 36, 217–223.

Bermejo, L. M., B. López-Plaza, C. Santurino, I. Cavero-Redondo, and C. Gómez-Candela. 2019. Milk and dairy product consumption and bladder cancer risk: a systematic review and meta-analysis of observational studies. Advances in Nutrition 10(suppl_2):S224-S238.

Bersaglieri, T., Sabeti, P.C., Patterson, N., Vanderploeg, T., Schaffner, S.F., Drake, J.A., Rhodes, M., Reich, D.E. and J.N. Hirschhorn. 2004. Genetic signatures of strong recent positive selection at the lactase gene. Am. J. Hum. Genet. 74:1111-1120.

Black, R. E., C. G. Victora, S. P. Walker, Z. A. Bhutta, P. Christian, M. de Onis, M. Ezzati, S. Grantham-McGregor, J. Katz, R. Martorell, and R. Uauy. 2013. Maternal and child undernutrition and overweight in low-income and middle-income countries. The Lancet 382(9890):427-451.

Bouvard, V., D. Loomis, K. Z. Guyton, Y. Grosse, F. E. Ghissassi, L. Benbrahim-Tallaa, N. Guha, H. Mattock, K. Straif, and D. Corpet. 2015. Carcinogenicity of consumption of red and processed meat. The Lancet Oncology 16(16):1599-1600.

Calle, E. E. and R. Kaaks. 2004. Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. Nature Reviews Cancer 4(8):579-591.

Castelli, W.P. 1988. Cholesterol and lipids in the risk of coronary artery disease--the Framingham Heart Study. Can. J. Cardiol. 4:5A-10A.

Chen, G.C., Szeto, I.M., Chen, L.H., Han, S.F., Li, Y.J., Van Hekezen, R. and L.Q. Qin. 2015. Dairy products consumption and metabolic syndrome in adults: systematic review and meta-analysis of observational studies. Sci. Rep. 5:14606.

Chowdhury, R., Warnakula, S., Kunutsor, S., Crowe, F., Ward, H.A., Johnson, L., Franco, O.H., Butterworth, A.S., Forouhi, N.G., Thompson, S.G. and K.T. Khaw. 2014. Association of dietary,

circulating, and supplement fatty acids with coronary risk: a systematic review and meta-analysis. Ann. Intern. Med. 160:398-406.

Curry, A., 2013. The milk revolution. Nature 500:20.

Data world bank. 2020. UNICEF, WHO, World Bank: Joint child malnutrition estimates. https://data.worldbank.org/indicator/SH.STA.STNT.ZS?end=2019&start=1983&view=chart&year=1983

Dayton, S. and M. L. Pearce. 1969. Prevention of coronary heart disease and other complications of arteriosclerosis by modified diet. Am J Med 46(5):751-762.

De Heinzelin, J., J. D. Clark, T. White, W. Hart, P. Renne, G. WoldeGabriel, Y. Beyene, and E. Vrba. 1999. Environment and behavior of 2.5-million-year-old Bouri hominids. Science 284(5414):625-629.

de Onis, M. and F. Branca. 2016. Childhood stunting: a global perspective. Maternal & Child Nutrition 12(S1):12-26.

De Souza, R.J., Mente, A., Maroleanu, A., Cozma, A.I., Ha, V., Kishibe, T., Uleryk, E., Budylowski, P., Schünemann, H., Beyene, J. and S.S. Anand. 2015. Intake of saturated and trans unsaturated fatty acids and risk of all cause mortality, cardiovascular disease, and type 2 diabetes: systematic review and meta-analysis of observational studies. Bmj. 351p.h3978.

Dehghan, M., Mente, A., Rangarajan, S., Sheridan, P., Mohan, V., Iqbal, R., Gupta, R., Lear, S., Wentzel-Viljoen, E., Avezum, A. and P. Lopez-Jaramillo. 2018. Association of dairy intake with cardiovascular disease and mortality in 21 countries from five continents (PURE): a prospective cohort study. The Lancet. https://doi.org/10.1016/S0140-6736(18)31812-9

Després, J.-P., B. Lamarche, P. Mauriège, B. Cantin, G. R. Dagenais, S. Moorjani, and P.-J. Lupien. 1996. Hyperinsulinemia as an independent risk factor for ischemic heart disease. New England Journal of Medicine 334(15):952-958.

doi: 10.1080/17512433.2018.1519391.

Dong, J.-Y., L. Zhang, K. He, and L.-Q. Qin. 2011. Dairy consumption and risk of breast cancer: a meta-analysis of prospective cohort studies. Breast cancer research and treatment 127(1):23-31.

Elwood, P.C., Givens, D.I., Beswick, A.D., Fehily, A.M., Pickering, J.E. and J. Gallacher. 2008. The survival advantage of milk and dairy consumption: an overview of evidence from cohort studies of vascular diseases, diabetes and cancer. J. Am. Coll. Nutr. 27:723S-734S.

Esposito, K., Chiodini, P., Colao, A., Lenzi, A. and D. Giugliano. 2012. Metabolic syndrome and risk of cancer: a systematic review and meta-analysis. Diabetes Care 35:2402–2411

Fogelholm, M., N. Kanerva, and S. Männistö. 2015. Association between red and processed meat consumption and chronic diseases: the confounding role of other dietary factors. European journal of clinical nutrition 69(9):1060-1065.

Foote, M. R., S. L. Giesy, G. Bernal-Santos, D. E. Bauman, and Y. R. Boisclair. 2010. t10,c12-CLA decreases adiposity in peripubertal mice without dose-related detrimental effects on mammary development, inflammation status and metabolism. Am. J. Physiol. Regul. Integr. Comp. Physiol. 299:R1521-1528.

Forouhi, N.G., Koulman, A., Sharp, S.J., Imamura, F., Kröger, J., Schulze, M.B., Crowe, F.L., Huerta, J.M., Guevara, M., Beulens, J.W. and G.J. van Woudenbergh. 2014. Differences in the prospective association between individual plasma phospholipid saturated fatty acids and incident type 2 diabetes: the EPIC-InterAct case-cohort study. Lancet Diabetes Endocrinol. 2:810-818.

García, R. G., M. Y. Rincón, W. D. Arenas, S. Y. Silva, L. M. Reyes, S. L. Ruiz, F. Ramirez, P. A. Camacho, C. Luengas, and J. F. Saaibi. 2011. Hyperinsulinemia is a predictor of new cardiovascular events in Colombian patients with a first myocardial infarction. International journal of cardiology 148(1):85-90.

Gaucheron, F. 2005. The minerals of milk. Reprod. Nutr. Dev. 45: 473-483.

Guyenet, S. J. and M. W. Schwartz. 2012. Regulation of Food Intake, Energy Balance, and Body Fat Mass: Implications for the Pathogenesis and Treatment of Obesity. The Journal of Clinical Endocrinology & Metabolism 97(3):745-755.

Hall, K. D., A. Ayuketah, R. Brychta, H. Cai, T. Cassimatis, K. Y. Chen, S. T. Chung, E. Costa, A. Courville, and V. Darcey. 2019. Ultra-processed diets cause excess calorie intake and weight gain: an inpatient randomized controlled trial of ad libitum food intake. Cell metabolism 30(1):67-77. e63.

Hamley, S. 2017. The effect of replacing saturated fat with mostly n-6 polyunsaturated fat on coronary heart disease: a meta-analysis of randomised controlled trials. Nutr J 16(1):30.

Harcombe, Z., Baker, J.S., Cooper, S.M., Davies, B., Sculthorpe, N., DiNicolantonio, J.J. and f. Grace. 2015. Evidence from randomised controlled trials did not support the introduction of dietary fat guidelines in 1977 and 1983: a systematic review and meta-analysis. Open heart 2:p.e000196.

Health Canada. 2019. Canada's dietary guidelines for health profesionals and policy makers. Canada.ca/Foodguide.

Hegsted, D.M., R. B. McGandy, M. L. Myers, and F.J. Stare. 1965. Quantitative effects of dietary fat on serum cholesterol in man. Am. J. Clin. Nutr. 17:281-295.

Heileson, J. L. 2019. Dietary saturated fat and heart disease: a narrative review. Nutrition Reviews.

Holt, P. R. 1999. Dairy foods and prevention of colon cancer: human studies. Journal of the American College of Nutrition 18(sup5):379S-391S.

Holt, P. R., E. O. Atillasoy, J. Gilman, J. Guss, S. F. Moss, H. Newmark, K. Fan, K. Yang, and M. Lipkin. 1998. Modulation of Abnormal Colonic Epithelial Cell Proliferation and Differentiation by Low-Fat Dairy FoodsA Randomized Controlled Trial. JAMA 280(12):1074-1079.

Huncharek, M., J. Muscat, and B. Kupelnick. 2008. Colorectal cancer risk and dietary intake of calcium, vitamin D, and dairy products: a meta-analysis of 26,335 cases from 60 observational studies. Nutrition and cancer 61(1):47-69.

Jenkins, T. C., R. J. Wallace, P. J. Moate, and E. E. Mosley. 2008. Board-invited review: Recent advances in biohydrogenation of unsaturated fatty acids within the rumen microbial ecosystem. J. Anim. Sci. 86(2):397-412.

Jensen, M.D., Ryan, D.H., Apovian, C.M., Ard, J.D., Comuzzie, A.G., Donato, K.A., Hu, F.B., Hubbard, V.S., Jakicic, J.M., Kushner, R.F. and C.M. Loria. 2014. 2013 AHA/ACC/TOS guideline for the management of overweight and obesity in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and The Obesity Society. J. Am. Coll. Cardiol. 63:2985-3023.

Jensen, R. G. 2002. The composition of bovine milk lipids: January 1995 to December 2000. J. Dairy Sci. 85(2):295-350.

Jeyaraman, M. M., Abou-Setta, A. M., Grant, L., Farshidfar, F., Copstein, L., Lys, J., ... & Zarychanski, R. (2019). Dairy product consumption and development of cancer: an overview of reviews. BMJ open, 9(1), bmjopen-2018.

Johnson, R.K., Appel, L.J., Brands, M., Howard, B.V., Lefevre, M., Lustig, R.H., Sacks, F., Steffen, L.M. and J. Wylie-Rosett. 2009. Dietary sugars intake and cardiovascular health: a scientific statement from the American Heart Association. Circulation 120:1011-1020.

Johnston, B. C., D. Zeraatkar, M. A. Han, R. W. Vernooij, C. Valli, R. El Dib, C. Marshall, P. J. Stover, S. Fairweather-Taitt, and G. Wójcik. 2019. Unprocessed red meat and processed meat consumption: dietary guideline recommendations from the Nutritional Recommendations (NutriRECS) Consortium. Ann Intern Med 171:756-764.

Kannel, W.B., Castelli, W.P. and Gordon, T., 1979. Cholesterol in the prediction of atherosclerotic disease: new perspectives based on the Framingham Study. Ann. Intern. Med. 90:85-91.

Kannel, W.B., Dawber, T.R., Kagan, A., Revotskie, N. and Stokes, J., 1961. Factors of risk in the development of coronary heart disease—six-year follow-up experience: the Framingham Study. Ann. Intern. Med. 55:33-50.

Kennedy, A., K. Martinez, S. Schmidt, S. Mandrup, K. LaPoint, and M. McIntosh. 2010. Antiobesity mechanisms of action of conjugated linoleic acid. The Journal of nutritional biochemistry 21(3):171-179.

Keys, A., 1953. Atherosclerosis: a problem in newer public health. Atherosclerosis 1:19.

Keys, A., Aravanis, C., Blackburn, H.W., Van Buchem, F.S., Buzina, R., Djordjevic, B.D., Dontas, A.S., Fidanza, F., Karvonen, M.J., Kimura, N. and D. Lekos. 1966. Epidemiological studies related to coronary heart disease: characteristics of men aged 40-59 in seven countries. Acta Med. Scand. Suppl. 460:1–392.

Konstantinov, I.E., Mejevoi, N. and Anichkov, N.M., 2006. Nikolai N. Anichkov and his theory of atherosclerosis. Tex. Heart Inst. J. 33:417.

Krauss, R.M., 2005. Dietary and genetic probes of atherogenic dyslipidemia. Arterioscler. Thromb. Vasc. Biol. 25:2265-2272.

Kruth, H. S. 2001. Lipoprotein cholesterol and atherosclerosis. Curr. Mol. Med. 1:633-653.

Kuzdzal-Savoie, S. 1964. Comparison of branched chain fatty acids in the feed and the milk of cows. Pages 287-294 in Proc. Annales de Biologie Animale, Biochimie, Biophysique.

Lamarche, B., A. Tchernof, S. Moorjani, B. Cantin, G. R. Dagenais, P. J. Lupien, and J.-P. Després. 1997. Small, dense low-density lipoprotein particles as a predictor of the risk of ischemic heart disease in men: prospective results from the Qué bec Cardiovascular Study. Circulation 95(1):69-75.

Lefevre, M., Champagne, C.M., Tulley, R.T., Rood, J.C. and M.M. Most. 2005. Individual variability in cardiovascular disease risk factor responses to low-fat and low-saturated-fat diets in men: body mass index, adiposity, and insulin resistance predict changes in LDL cholesterol–. Am. J. Clin. Nut. 82:957-963.

Leren, P. 1970. The Oslo diet-heart study. Eleven-year report. Circulation 42(5):935-942.

Liebert, A., López, S., Jones, B.L., Montalva, N., Gerbault, P., Lau, W., Thomas, M.G., Bradman, N., Maniatis, N. and D.M. Swallow. 2017. World-wide distributions of lactase persistence alleles and the complex effects of recombination and selection. Hum. Genet. 136:1445-1453.

Lock, A. L. and D. E. Bauman. 2004. Modifying milk fat composition of dairy cows to enhance fatty acids beneficial to human health. Lipids 39(12):1197-1206.

López-Plaza, B., L. M. Bermejo, C. Santurino, I. Cavero-Redondo, C. Álvarez-Bueno, and C. Gómez-Candela. 2019. Milk and Dairy Product Consumption and Prostate Cancer Risk and Mortality: An Overview of Systematic Reviews and Meta-analyses. Adv Nutr 10(suppl_2):S212-S223.

Lustig, R. H. 2010. Fructose: Metabolic, hedonic, and societal parallels with ethanol. J. Am. Diet. Assoc.110:1307-1321.

Luz, P. L. D., Favarato, D., Faria-Neto Junior, J. R., Lemos, P., and A.C.P. Chagas. 2008. High ratio of triglycerides to HDL-cholesterol predicts extensive coronary disease. Clinics 63:427-432.

Mao, Q.-Q., Y. Dai, Y.-W. Lin, J. Qin, L.-P. Xie, and X.-Y. Zheng. 2011. Milk Consumption and Bladder Cancer Risk: A Meta-Analysis of Published Epidemiological Studies. Nutrition and Cancer 63(8):1263-1271.

Mensink, R.P., Zock, P.L., Kester, A.D. and M.B. Katan. 2003. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. Am. J. Clin. Nut. 77:1146-1155.

Micha, R. and D. Mozaffarian. 2008. Trans fatty acids: effects on cardiometabolic health and implications for policy. Prostaglandins Leukot. Essent. Fatty Acids 79:147-152.

Missmer, S. A., S. A. Smith-Warner, D. Spiegelman, S.-S. Yaun, H.-O. Adami, W. L. Beeson, P. A. Van Den Brandt, G. E. Fraser, J. L. Freudenheim, and R. A. Goldbohm. 2002. Meat and dairy food consumption and breast cancer: a pooled analysis of cohort studies. International journal of epidemiology 31(1):78-85.

Monette, J.S., Hutchins, P.M., Ronsein, G.E., Wimberger, J., Irwin, A.D., Tang, C., Sara, J.D., Shao, B., Vaisar, T., Lerman, A. and J.W. Heinecke. 2016. Patients with coronary endothelial dysfunction have impaired cholesterol efflux capacity and reduced HDL particle concentration. Circ. Res. 119:83-90.

Mozaffarian, D., 2014. Saturated fatty acids and type 2 diabetes: more evidence to re-invent dietary guidelines. Lancet Diabetes Endocrinol. 2:770-772.

Mozaffarian, D., Benjamin, E.J., Go, A.S., Arnett, D.K., Blaha, M.J., Cushman, M., Das, S.R., de Ferranti, S., Després, J.P., Fullerton, H.J. and V.J. Howard. 2015. Heart disease and stroke statistics—2016 update: a report from the American Heart Association. Circulation CIR-0000000000000350.

Mozaffarian, D., Cao, H., King, I.B., Lemaitre, R.N., Song, X., Siscovick, D.S. and G.S. Hotamisligil. 2010. Trans-palmitoleic acid, metabolic risk factors, and new-onset diabetes in US adults: a cohort study. Ann. Intern. Med. 153:790-799.

MRC. 1968. Controlled trial of soya-bean oil in myocardial infarction. Lancet 2(7570):693-699.

Nelson, G., J. Bogard, K. Lividini, J. Arsenault, M. Riley, T. B. Sulser, D. Mason-D'Croz, B. Power, D. Gustafson, and M. Herrero. 2018. Income growth and climate change effects on global nutrition security to mid-century. Nature Sustainability 1(12):773-781.

Norris, G. H. and C. N. Blesso. 2017. Dietary sphingolipids: Potential for management of dyslipidemia and nonalcoholic fatty liver disease. Nutrition reviews 75(4):274-285.

O'Donnell-Megaro, A.M., Barbano, D.M. and Bauman, D.E., 2011. Survey of the fatty acid composition of retail milk in the United States including regional and seasonal variations. J. Dairy Sci. 94:59-65.

Oomen, C.M.,Ocké, M.C., Feskens, E.J., van Erp-Baart, M.A.J., Kok, F.J. and Kromhout, D., 2001. Association between trans fatty acid intake and 10-year risk of coronary heart disease in the Zutphen Elderly Study: a prospective population-based study. The Lancet 357:746-751.

Ordovas, J.M. 2005. Diet-heart hypothesis: will diversity bring reconciliation?. Am J Clin Nutr. 82(5):919-20.

Park, Y.W. ed., 2009. *Bioactive components in milk and dairy products*. John Wiley & Sons.

Parodi, P. 2009. Milk fat nutrition. Pages 28-51. Wiley Online Library.

Parodi, P.W., 2009. Has the association between saturated fatty acids, serum cholesterol and coronary heart disease been over emphasized?. Int. Dairy J. 19:345-361.

Pierre, F., R. Santarelli, S. Taché, F. Guéraud, and D. E. Corpet. 2008. Beef meat promotion of dimethylhydrazine-induced colorectal carcinogenesis biomarkers is suppressed by dietary calcium. British Journal of Nutrition 99(5):1000-1006.

Pimpin, L., Wu, J.H., Haskelberg, H., Del Gobbo, L. and D. Mozaffarian. 2016. Is butter back? A systematic review and meta-analysis of butter consumption and risk of cardiovascular disease, diabetes, and total mortality. PLoS One 11:p.e0158118.

Poortvliet, P. C., S. Bérubé-Parent, V. Drapeau, B. Lamarche, J. E. Blundell, and A. Tremblay. 2007. Effects of a healthy meal course on spontaneous energy intake, satiety and palatability. British Journal of Nutrition 97(3):584-590.

Prentice, A.M., 2014. Dairy products in global public health–. Am. J. Clin. Nutr. 99:1212S-1216S.

Rachid, M., C. Matar, J. Duarte, and G. Perdigon. 2006. Effect of milk fermented with a Lactobacillus helveticus R389(+) proteolytic strain on the immune system and on the growth of 4T1 breast cancer cells in mice. FEMS Immunology & Medical Microbiology 47(2):242-253.

Ramsden, C.E., Zamora, D., Majchrzak-Hong, S., Faurot, K.R., Broste, S.K., Frantz, R.P., Davis, J.M., Ringel, A., Suchindran, C.M. and J.R. Hibbeln. 2016. Re-evaluation of the traditional diet-heart hypothesis: analysis of recovered data from Minnesota Coronary Experiment (1968-73). Bmj. 353:1246.

Ran-Ressler, R. R., S. Bae, P. Lawrence, D. H. Wang, and J. T. Brenna. 2014. Branched-chain fatty acid content of foods and estimated intake in the USA. British journal of nutrition 112(4):565-572.

Rautiainen, S., Wang, L., Lee, I.M., Manson, J.E., Buring, J.E. and H.D. Sesso. 2016. Dairy consumption in association with weight change and risk of becoming overweight or obese in middle-aged and older women: a prospective cohort study–3. Am. J. Clin. Nutr. 103:979-988.

Ravnskov, U., de Lorgeril, M., Diamond, D.M., Hama, R., Hamazaki, T., Hammarskjöld, B., Hynes, N., Kendrick, M., Langsjoen, P.H., Mascitelli, L. and K.S. McCully. 2018. LDL-C Does

Not Cause Cardiovascular Disease: a comprehensive review of current literature. Expert. Rev. Clin. Pharmacol. [Epub ahead of print].

Rice, B.H., Cifelli, C.J., Pikosky, M.A. and G.D. Miller. 2011. Dairy components and risk factors for cardiometabolic syndrome: recent evidence and opportunities for future research. Adv. Nutr. 2:396-407.

Rico, D. E., S. H. Preston, J. M. Risser, and K. J. Harvatine. 2015. Rapid changes in key ruminal microbial populations during the induction of and recovery from diet-induced milk fat depression in dairy cows. The British journal of nutrition 114(3):358-367.

Rohatgi, A., Khera, A., Berry, J.D., Givens, E.G., Ayers, C.R., Wedin, K.E., Neeland, I.J., Yuhanna, I.S., Rader, D.R., de Lemos, J.A. and P.W. Shaul. 2014. HDL cholesterol efflux capacity and incident cardiovascular events. N. Engl. J. Med. 371:2383-2393.

Sachdeva, A., Cannon, C.P., Deedwania, P.C., LaBresh, K.A., Smith Jr, S.C., Dai, D., Hernandez, A. and G.C. Fonarow. 2009. Lipid levels in patients hospitalized with coronary artery disease: an analysis of 136,905 hospitalizations in Get With The Guidelines. Am. Heart J. 157:111-117.

Sacks, F. M., A. H. Lichtenstein, J. H. Wu, L. J. Appel, M. A. Creager, P. M. Kris-Etherton, M. Miller, E. B. Rimm, L. L. Rudel, and J. G. Robinson. 2017. Dietary fats and cardiovascular disease: a presidential advisory from the American Heart Association. Circulation 136(3):e1-e23.

Saely, C. H., Rein, P. and H. Drexel. 2007. The metabolic syndrome and risk of cardiovascular disease and diabetes: experiences with the new diagnostic criteria from the International Diabetes Federation. Horm. Metab. Res. 39:642–650.

Saliba, L., R. Gervais, Y. Lebeuf, and P. Y. Chouinard. 2014. Effect of feeding linseed oil in diets differing in forage to concentrate ratio: 1. Production performance and milk fat content of biohydrogenation intermediates of α-linolenic acid. Journal of Dairy Research 81(1):82-90.

Siri-Tarino, P.W., Sun, Q., Hu, F.B. and R.M. Krauss. 2010. Meta-analysis of prospective cohort studies evaluating the association of saturated fat with cardiovascular disease. Am. J. Clin. Nutr. 91:535-546.

Smilowitz, J. T., Dillard, C. J., and J.B. German. 2005. Milk beyond essential nutrients: the metabolic food. Aust. J. Dairy Technol. 60:77.

Soedamah-Muthu, S.S., Ding, E.L., Al-Delaimy, W.K., Hu, F.B., Engberink, M.F., Willett, W.C. and J.M. Geleijnse. 2010. Milk and dairy consumption and incidence of cardiovascular diseases and all-cause mortality: dose-response meta-analysis of prospective cohort studies–. Am. J. Clin. Nutr. 93:158-171.

Statistics Canada. Food available in Canada; Table: 32-10-0054-01.
https://www150.statcan.gc.ca/t1/tbl1/en/tv.action?pid=3210005401

Stone, N.J., Robinson, J.G., Lichtenstein, A.H., Merz, C.N.B., Blum, C.B., Eckel, R.H., Goldberg, A.C., Gordon, D., Levy, D., Lloyd-Jones, D.M. and P. McBride. 2014. 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. J. Am Coll. Cardiol. 63:2889-2934.

Thompson, J. C., S. Carvalho, C. W. Marean, and Z. Alemseged. 2019. Origins of the human predatory pattern: The transition to large-animal exploitation by early hominins. Current Anthropology 60(1).

Turpeinen, O., M. J. Karvonen, M. Pekkarinen, M. Miettinen, R. Elosuo, and E. Paavilainen. 1979. Dietary prevention of coronary heart disease: the Finnish Mental Hospital Study. Int J Epidemiol 8(2):99-118.

Ulbricht, T.L.V. and D.A.T, Southgate. 1991. Coronary heart disease: seven dietary factors. The Lancet 338:985-992.

Ulven, S. M., K. B. Holven, A. Gil, and O. D. Rangel-Huerta. 2019. Milk and dairy product consumption and inflammatory biomarkers: an updated systematic review of randomized clinical trials. Advances in Nutrition 10(suppl_2):S239-S250.

USDA/USDHHS. U.S. Department of Agriculture and U.S. Department of Health and Human Services. 2010. Dietary Guidelines for Americans, 2010. 7th Edition, Washington, DC: U.S. Government Printing Office, December 2010. http://www.cnpp.usda.gov/DGAs2010-PolicyDocument.htm

Vesper, H., E.-M. Schmelz, M. N. Nikolova-Karakashian, D. L. Dillehay, D. V. Lynch, and A. H. Merrill Jr. 1999. Sphingolipids in food and the emerging importance of sphingolipids to nutrition. The Journal of nutrition 129(7):1239-1250.

Victora, C. G., M. de Onis, P. C. Hallal, M. Blössner, and R. Shrimpton. 2010. Worldwide Timing of Growth Faltering: Revisiting Implications for Interventions. Pediatrics 125(3):e473-e480.

Villeneuve, M.-P., Y. Lebeuf, R. Gervais, G. Tremblay, J. Vuillemard, J. Fortin, and P. Chouinard. 2013. Milk volatile organic compounds and fatty acid profile in cows fed timothy as hay, pasture, or silage. J. Dairy Sci. 96(11):7181-7194.

Wang, Y., and S. Li. 2008. Worldwide trends in dairy production and consumption and calcium intake: is promoting consumption of dairy products a sustainable solution for inadequate calcium intake?. Food Nutr. Bull. 29:172-185.

Willett, W.C. 2012. Dietary fats and coronary heart disease. J. Intern. Med. 272:13-24.

Windaus A. 1910. Ueber der Gehalt normaler und atheromatoser Aorten an Cholesterol und Cholesterinester. Zeitschrift. Physiol. Chemie. 67:174.

Wongtangtintharn, S., H. Oku, H. Iwasaki, and T. Toda. 2004. Effect of branched-chain fatty acids on fatty acid biosynthesis of human breast cancer cells. Journal of Nutritional Science and Vitaminology 50(2):137-143.

Wu, S. H., Liu, Z. and S.C. Ho. Metabolic syndrome and all-cause mortality: a meta-analysis of prospective cohort studies. Eur. J. Epidemiol. 25:375–384.

Yakoob, M.Y., Shi, P., Hu, F.B., Campos, H., Rexrode, K.M., Orav, E.J., Willett, W.C. and D. Mozaffarian. 2014. Circulating biomarkers of dairy fat and risk of incident stroke in US men and women in 2 large prospective cohorts–. Am. J. Clin. Nutr. 100: 1437-1447.

Yanagi, S., Yamashita, M. and Imai, S. (1993) Sodium butyrate inhibits the enhancing effect of high fat diet on mammary tumorigenesis. Oncology:50, 201–204.

Yang, Q., Zhang, Z., Gregg, E.W., Flanders, W.D., Merritt, R. and F.B. Hu. 2014. Added sugar intake and cardiovascular diseases mortality among US adults. JAMA Intern. Med. 174:516-524.

Yang, Z., S. Liu, X. Chen, H. Chen, M. Huang, and J. Zheng. 2000. Induction of apoptotic cell death and in vivo growth inhibition of human cancer cells by a saturated branched-chain fatty acid, 13-methyltetradecanoic acid. Cancer research 60(3):505-509.

Yerushalmy, J. and H.E. Hilleboe. 1957. Fat in the diet and mortality from heart disease; a methodologic note. N. Y. State J Med. 57:2343.

Young, S.S. and A. Karr. 2011. Deming, data and observational studies. Significance, 8:116-120.

Zang, J., M. Shen, S. Du, T. Chen, and S. Zou. 2015. The association between dairy intake and breast cancer in western and Asian populations: a systematic review and meta-analysis. Journal of breast cancer 18(4):313-322.

Phosphorus utilization of livestock: Challenges and approaches for improvement

Utilisation du phosphore par le bétail : défis et approches pour l'amélioration

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Abstract

All living organisms depend on a continuous supply of phosphorus (P). Meeting the P requirements of livestock through a mix of P sources is mandatory for both, animal health and performance. However, finite global rock phosphate is the main basis of feed phosphate production and P excretion of animals can be harmful to the environment in areas with high livestock density. Hence, becoming independent of rock phosphate resources is a big challenge for improving sustainability of agricultural production. One action to be taken is that dietary P allowances are regularly updated and diversified – across all livestock species. Other handling options depend on animal species and their ability to utilize phytate-P from plant-based feed materials. In cattle and other ruminants, microbial activity in the rumen cleaves P from phytate. While recent studies indicated that the kinetic parameters of ruminal phytate degradation are different among feedstuffs, phytate-P utilization of ruminants overall is high. However, P oversupply – even in diets without mineral P supplement – may result from using oilseed meals as a primary protein source in diets of cattle. In pigs and poultry, application of phase-feeding and adjustment of digestible P concentrations in the diet to growth phases is effective in sparing P. Supplementing phytase also is effective through increased digestibility of phytate-P. While the current trend is to apply higher phytase levels than before, some phytate-P remains undigested in pigs and poultry. The adjustment of dietary Ca and P supplements and future enzyme engineering may help in making more phytate-P available to nonruminants.

Résumé

Tous les organismes vivants dépendent d'un apport continu de phosphore (P). Répondre aux besoins en P du bétail par un mélange de sources de P est indispensable pour maintenir la santé et les performances des animaux. Toutefois, le phosphate naturel, dont la quantité est limitée à l'échelle mondiale, est la principale base de la production de phosphate alimentaire, et l'excrétion de P par les animaux peut être nocive pour l'environnement dans les zones à forte densité de bétail. Par conséquent, s'affranchir de la dépendance aux ressources en phosphate naturel est un grand défi à relever pour améliorer le caractère durable de la production agricole. Une des mesures à prendre consiste à mettre à jour et à diversifier régulièrement les apports en P alimentaire, et ce pour toutes les espèces animales. D'autres possibilités dépendent de l'espèce animale en question et de sa capacité à utiliser le P sous forme de phytates provenant de matières premières végétales. Chez les bovins et les autres ruminants, l'activité microbienne dans le rumen permet de séparer le P des phytates. Alors que des études récentes ont indiqué que les paramètres cinétiques de la dégradation des phytates dans le rumen sont différents d'un aliment à l'autre, l'utilisation du P des phytates chez les ruminants est globalement élevée. Cependant,

l'usage de farines de graines oléagineuses comme source primaire de protéines dans les régimes des bovins peut entraîner un excès de P, même dans les régimes sans supplément de P minéral. Chez les porcs et les volailles, l'adoption d'une alimentation par phases et l'adaptation des concentrations de P digestible dans le régime aux différentes phases de croissance sont efficaces pour diminuer l'excrétion de P. La supplémentation en phytase est également utile dans la mesure où elle accroît la digestibilité du P des phytates. Bien que la tendance actuelle soit d'utiliser des concentrations de phytase plus élevées qu'auparavant, une partie du P des phytates demeure non digérée chez les porcs et les volailles. L'adaptation des suppléments alimentaires de Ca et P et les progrès à venir du génie enzymatique pourraient contribuer à augmenter la disponibilité du P des phytates pour les non-ruminants.

Introduction

All living organisms depend on the continuous supply of available phosphorus (P) sources for a plethora of metabolic processes. Fertilizer and feed phosphates are obtained to a large extent from rock phosphate. This is a non-renewable resource, and the global distribution of rock phosphate reserves is not uniform. Concurrently, P accumulation in areas with high livestock production density may be harmful to the environment. Hence, saving P resources for future generations is an important objective for all participants along the food chain and likely one of the greatest challenges for the sustainability of agricultural production systems (Gross, 2010; Neset and Cordell, 2012). For the livestock industries, this indicates a need for further improvement in the efficiency of P utilization and coupling of animal production size and manure P produced with the available arable land.

In livestock feeding, most of the P supplied originates from plant seeds (such as cereal and leguminous grains) and by-products of the food and energy sector, such as oilseed meals, press cakes, brans, and dried distillers' grains. In the vast majority of these plant-based feeds, more than half of the P is contained in the form of phytic acid and its salt, phytate ($InsP_6$). Swine and birds are more restricted in their digestive capacity to release P from $InsP_6$ than ruminants. Hence, mineral feed phosphates are often added to feed to meet the P requirement of animals. This is of special relevance in regions such as the European Union, where animal proteins (including P contained therein) are not allowed to be used in animal feeding (Rodehutscord et al., 2002). Using mineral feed phosphates may not be sustainable, particularly when feed phosphates are manufactured from rock phosphates. Different approaches are of interest and need to be combined in an attempt to further increase the efficiency of P utilization in livestock industries. These are related to an increase in the digestibility of P bound in the form $InsP_6$ ($InsP_6$-P), revision and diversification of dietary P allowances, and attempts to keep P cycles closed (again) in different segments of production. Challenges and approaches are different in ruminant and nonruminant species, which is why they are separately addressed herein.

Broiler Chickens

In recent years, the process of $InsP_6$ degradation in the digestive tract and factors influencing dephosphorylation have been extensively studied in broiler chickens. It had been presumed for a long time that endogenous $InsP_6$ degradation (hydrolysis by mucosal or microbial phosphatases) in the digestive tract of poultry plays a little role, if any. However, several studies have shown that this presumption may not be correct. Literature in this field has recently been reviewed elsewhere (Rodehutscord, 2017), and the following provides a brief summary. When broiler chickens are challenged by a low supply of P and calcium (Ca), for instance by removal of feed phosphates from the diet, up to two-thirds of the $InsP_6$ contained in the diet is degraded, and the released phosphate is absorbed throughout the small intestine, despite the diet not containing a phytase supplement. This degradation is catalyzed by phytases and other phosphatases provided by epithelial cells or microorganisms in the digestive tract. In collaboration with colleagues from the University of Saskatchewan, we found that endogenous epithelial enzymes may be more important in $InsP_6$ degradation than microbial enzymes (Sommerfeld et al., 2019). When mineral P sources are added to the diet, which is very common in the poultry industry, endogenous $InsP_6$ degradation by broilers is reduced by approximately 0.5 g for each gram of mineral P supplemented (Figure 1). This effect is even more pronounced when Ca is included in the supplement and especially when Ca is supplemented in excess of that required (Sommerfeld et al., 2018a). When phytase is added to the feed, it can compensate for the diminishing effects of mineral P and Ca on $InsP_6$ degradation.
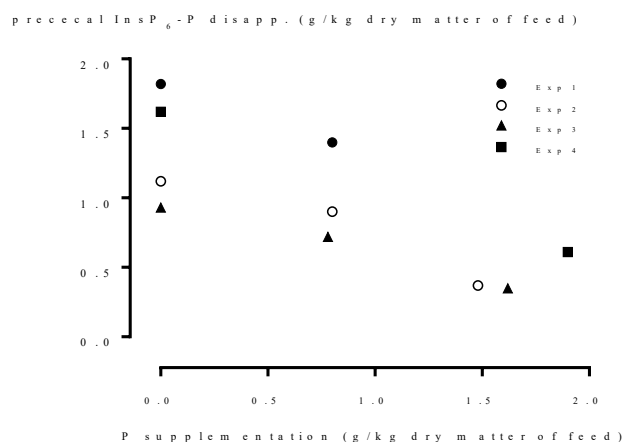
Figure 1. Effects of mineral P supplements on the prececal disappearance of $InsP_6$-P in studies conducted with broiler chickens (Shastak et al., 2014; Zeller et al., 2015b; Sommerfeld et al., 2019). The mean of the estimated slopes is -0.48.

The development and application of phytase as a feed additive contributes to making the nonruminant production sector more sustainable. Many recent studies have shown that phytase supplementation to broiler diets can increase gastrointestinal $InsP_6$ degradation to a large extent. In studies by our group using different types of diets, $InsP_6$ degradation, up to the terminal ileum, was up to 80-90 % (Zeller et al., 2015a; Sommerfeld et al., 2018a; Ingelmann et al., 2019) and even greater than 90 % when phytase supplementation exceeded 1500 FTU/kg of feed (Zeller et al., 2015b; Sommerfeld et al., 2018b; Siegert et al., 2019; Ajuwon et al., 2020). Accordingly, the potential to achieve further improvements in P digestibility by increasing $InsP_6$ accessibility above what is currently possible is not great. However, although some *myo*-inositol is released in the digestive tract upon phytase supplementation (Sommerfeld et al.,

2018b; Sommerfeld et al., 2018a), dephosphorylation of the inositol ring is not complete. Isomers of lower inositol phosphates, such as $InsP_4$ and $InsP_3$, are present at the terminal ileum of broilers when they were fed phytase-supplemented diets and these isomers cause approximately 20 % of the P contained in the initially hydrolyzed $InsP_6$ to be non-digestible by the broilers (Rodehutscord, 2017). If it were possible to overcome these limitations in the degradation cascade, further improvement in the utilization of $InsP_6$-P above the current values could be achieved.

Supplements of Ca sources, especially limestone, are well known to reduce the solubility and prececal degradation of $InsP_6$ with corresponding effects on P digestibility (Tamim and Angel, 2003). This effect seems to be specifically relevant when diets do not contain a phytase supplement, whereas there were no negative effects of monocalcium phosphate supplementation on $InsP_6$ degradation at very high levels of phytase supplementation (Zeller et al., 2015b). However, when 1500 FTU/kg of phytase was used in diets with an adequate Ca concentration, prececal $InsP_6$ degradation remained below the value achieved at a reduced Ca concentration (Sommerfeld et al., 2018a). Therefore, approaches targeting the maximum P digestibility in broilers by using phytase must avoid an excess Ca supply.

Dietary P allowances for broiler chickens
Several studies have targeted the P requirement of broiler chickens and factors affecting that requirement. Summarizing this research goes beyond the scope of this paper and may be impossible because of the differences in the main feed ingredients, P sources, interactions between mineral P, Ca, and phytate, response criteria, and the manner of expression. The age of the broilers is important, and the strain may also play a role. Nonetheless, some of the literature has been extracted following defined criteria and used for meta-analyses. Létourneau-Montminy et al. (2010) calculated how variation in Ca concentration and phytase supplementation affected the concentration of non-phytate-P needed in the diet for growth, gain-to-feed ratio, and tibia ash concentration of 3-week-old broilers. The results of the calculations were consistent with the aforementioned Ca effects on $InsP_6$ degradation and indicated that more non-phytate-P was needed in the diet when Ca concentration increased (Létourneau-Montminy et al., 2010; Faridi et al., 2015). Both meta-analyses showed that more P is needed for high bone mineralization than for growth or the gain-to-feed ratio. Such meta-analyses are very useful in generating data for optimizing diets but have a disadvantage in that they are related to predefined classes of growth or age.

Following the guidance of WPSA (2013), data on the requirement of prececal digestible P are needed. In agreement with this and as an alternative to meta-analyses, factorial approaches were suggested for use in poultry (Rodehutscord, 2006; Khaksar et al., 2017). This implies that single factors such as endogenous P loss and P accretion can be determined separately and used in flexible models. Such models can consider variables such as broiler strain and the P content of their body and its change with age, growth rate, and feed conversion ratio. Such a model of digestible P requirement has been suggested by Khaksar et al. (2017). When entry variables that represent broiler standards are chosen, the required digestible P concentration in the diet continuously decreases with increasing age (Figure 2). Figure 2 also shows how total P concentration is affected by dependence upon the P digestibility of the diet, irrespective of whether this is caused by the choice of feed raw materials, phytase supplementation, or both. Clearly, there is considerable potential to reduce P concentration in the diet with increasing age. The older the broilers are, the higher is the potential to save P resources. This is a combined effect of what is shown in Figure 2 and the fact that the feed-to-gain ratio increases with the broiler's age.
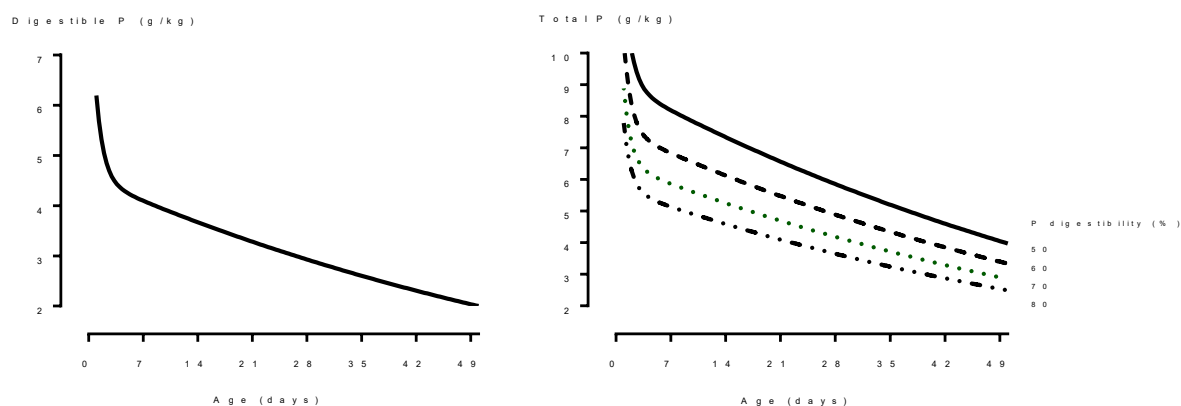
Figure 2. Course of digestible P and total P concentration needed in diets for broilers during growth. The left panel is based on the model of Khaksar et al. (2017) and feed intake curves of commercial broilers. The right panel shows resulting concentrations of total P dependent upon P digestibility of the diet.

Laying Hens

When compared to broiler chickens, the process and extent of InsP$_6$ degradation and affecting factors are not well understood in laying hens. In early work on this subject, principal responses of laying hens appeared similar to those characterized above for broiler chickens (Van der Klis et al., 1997). When corn-soybean meal-based diets were used, prececal InsP$_6$ degradation and P digestibility were significantly lower with 4 % Ca in the diet compared to that with 3 % Ca in the diet, but monocalcium phosphate did not affect InsP$_6$ degradation. Phytase supplementation (500 FTU/kg) increased prececal InsP$_6$ degradation up to 72 %, but the negative effect of Ca remained in the diets containing phytase. The response in prececal InsP$_6$ degradation was not reflected in performance and bone data of hens, which were considered indicators of overestimation of P requirement for laying hens. Phytase supplementation effects were also investigated using single ingredients as the only source of P in the diet of layers (Leske and Coon, 1999). The diets were provided with or without 300 FTU/kg phytase. InsP$_6$ degradation measured in excreta had increased from 26 % to 62 % in soybean meal, 23 % to 52 % in corn, and 4 % to 51 % in rice bran. A recent report showed that in the gizzard and ileum content in hens, concentrations of *myo*-inositol were significantly higher and those of InsP$_6$ and InsP$_5$ were lower when wheat-based diets were supplemented with 1500 FTU/kg phytase (Taylor et al., 2018). The authors did not report prececal InsP$_6$ degradation; however, prececal P digestibility was increased from 39 % to 70 % by 1500 FTU/kg phytase. The diets in this study had high Ca concentrations (> 4.4 %).

Dietary P allowances for laying hens
In a meta-analysis of data from 14 experiments with laying hens using corn-based diets, estimated non-phytate-P requirements ranged from 1.5 to 2.2 g/kg feed, depending on the chosen response trait and whether or not 300 FTU/kg phytase was supplemented (Ahmadi and Rodehutscord, 2012). Compared to the recommendations of different committees, this range is much lower. More recent studies confirmed that traditional recommendations overestimate the requirements of layers. In a 12-week study using several response criteria, the authors concluded that their data indicated that reduction to 1.5 g of "available" P is possible without affecting the health or performance of laying hens (Jing et al., 2018). Other authors suggested

that oversupply of P can reduce the digestibility of other nutrients (Hughes et al., 2009). Thus, the potential to save feed phosphates in laying hen feed is high.

Swine

Feedstuff evaluation in swine nutrition includes total tract P digestibility. Standard assays for the determination of P digestibility in growing swine, such as the one recommended by the Gesellschaft für Ernährungsphysiologie GfE (GfE, 1994), have been used for many years. Data determined using this assay show a wide range of P digestibility among feed raw materials for swine (Figure 3). The solubility of non-plant P sources and phytate content, as well as intrinsic plant phytase activity, are the main sources of variation.
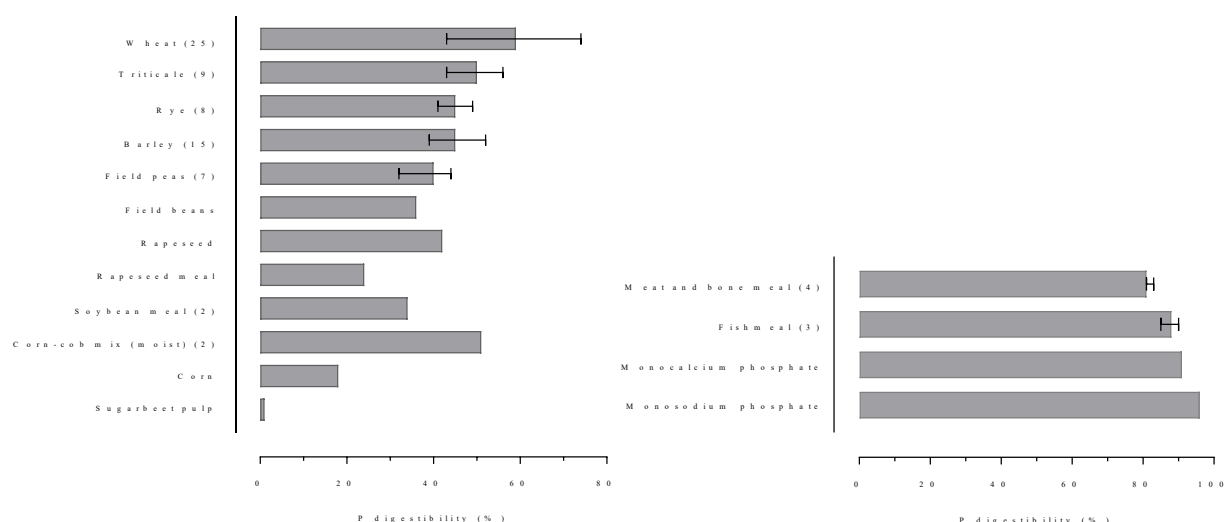
Figure 3. Total tract P digestibility of some feed raw materials for growing swine determined by using the standard assay of GfE (1994). Means and range from minimum and maximum values (if three or more batches per feed), values in parentheses are the number of tested batches of feed. Data from Düngelhoef et al., 1994; Rodehutscord et al., 1994; Rodehutscord et al., 1996; Krause et al., 1997; Rodehutscord et al., 1997; Hovenjürgen et al., 1999; Hovenjürgen et al., 2003; Kluth et al., 2004; Schemmer, 2020.

Unlike broiler chickens, $InsP_6$ degradation in the prececal section of the gastrointestinal tract of pigs is relatively low (Figure 4). This might result from mucosal phosphatase activities being lower in pigs than in broiler chickens.

prececal InsP$_6$ disappearance (%)

Means and 95% CI

Pigs (8 studies)   Broilers (10 studies)
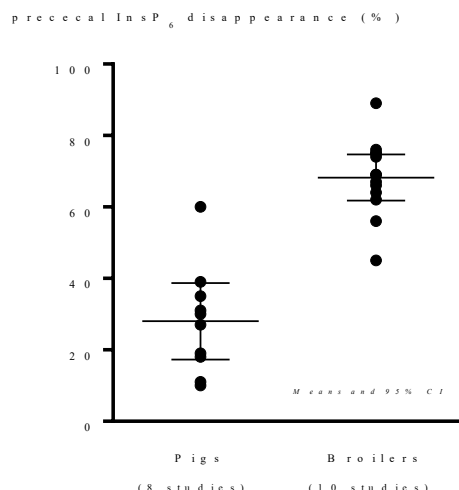
Figure 4. Disappearance of InsP$_6$ determined at the end of the ileum when corn-based low-P diets were fed without phytase supplement. Data from Jongbloed et al., 1992; Kemme et al., 1999; Rapp et al., 2001; Applegate et al., 2003; Tamim and Angel, 2003; Tamim et al., 2004; Kemme et al., 2006; Baumgärtel et al., 2008; Leytem et al., 2008; Rutherfurd et al., 2014; Shastak et al., 2014; Zeng et al., 2014; Zeller et al., 2015a; Zeller et al., 2015b; Sommerfeld et al., 2018a; Ingelmann et al., 2019; Siegert et al., 2019; Rosenfelder-Kuon et al., 2020b.

Irrespective of the amount of InsP$_6$ degraded prececally, InsP$_6$ was barely present in feces of pigs, indicating that post-ileal InsP$_6$ disappearance was nearly complete (Sandberg et al., 1993; Schlemmer et al., 2001; Baumgärtel et al., 2008; Rosenfelder-Kuon et al., 2020b). Because P absorption posterior to the ileum does not seem to be relevant, phosphate released from InsP$_6$ in the hindgut remains unavailable to the animal and is excreted in forms other than InsP$_6$. Consequently, total P digestibility in pigs, determined according to a standard protocol and using mash feed (GfE, 1994), barely exceeded 30 % in low-phytase feed such as corn or oilseed meals (Düngelhoef et al., 1994; Rodehutscord et al., 1996), whereas it was approximately 45 % in barley, 52 % in triticale, 68-73 % in rye, and 43-74 % in wheat (Düngelhoef et al., 1994; Rodehutscord et al., 1996; Hovenjürgen et al., 2003). Those cereals are known to contain intrinsic plant phytase activity.

Phytase is commonly added to diets for growing pigs to increase P digestibility and reduce the inclusion of mineral P sources. Despite high variation in the efficacy of phytase added to increase P digestibility, the overall upper limit of P digestibility appears to reach 65 %, according to a recent meta-analysis (Rosenfelder-Kuon et al., 2020a). The maximal increase in overall P digestibility with low-P diets because of phytase was 26 % points, a value not much different than that from a meta-analysis conducted in 1995 (Figure 5). Higher effect of increased phytase addition may be expected, especially because the disappearance of InsP$_6$ by the end of ileum can be up to 90 %. However, recent studies have shown that the degradation process is not complete. Some InsP$_4$ and other lower inositol phosphates remain undegraded at the terminal ileum, and approximately 20 % of InsP$_6$-P remains undigested in such a form (Mesina et al., 2018; Rosenfelder-Kuon et al., 2020b). Any attempt for further improvement in phytase efficacy should therefore focus on improving dephosphorylation of lower inositol phosphates instead of the initial dephosphorylation of InsP$_6$.
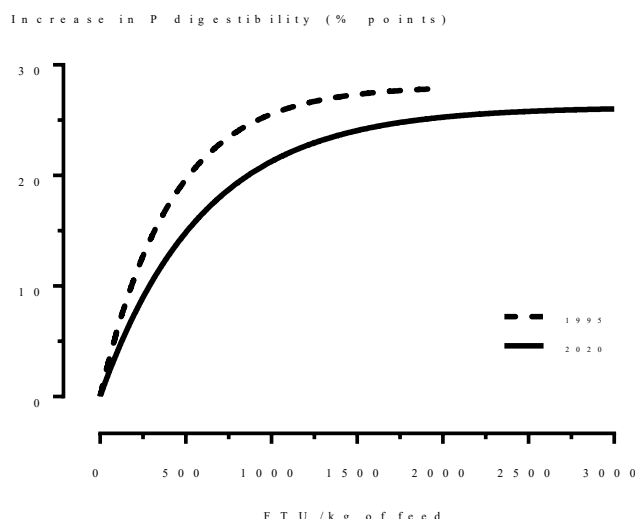
Figure 5. Predicted responses in P digestibility by pigs determined by two meta-analyses of data in the literature (Düngelhoef and Rodehutscord, 1995; Rosenfelder-Kuon et al., 2020a)

Ruminants

The microbiome in the forestomach shows remarkable phytase activity (Yanke et al., 1998) and provides the potential for almost complete phytate degradation in the rumen. This is related to a high absorbability of $InsP_6$-P in the small intestine. However, results for fecal disappearance of $InsP_6$ are inconsistent in the literature, and values range from 5 % to more than 20 % of the $InsP_6$ intake excreted with feces (Morse et al., 1992; Kincaid et al., 2005; Ray et al., 2013; Haese et al., 2014). Part of this variation may occur because of differences between $InsP_6$ sources, whereas others are caused by differences in performance, feed intake, and related differences in ruminal passage rate, causing variable exposure of dietary $InsP_6$ to microbial activity in the rumen. $InsP_6$ disappearance of rapeseed meal proceeded slowly compared to that of other feeds, such as soybean meal, corn, and wheat (Haese et al., 2017b; Haese et al., 2017a). *In situ* studies investigating $InsP_6$ disappearance from polyester bags in the rumen of rumen fistulated cows indicated distinct differences in $InsP_6$ degradation among concentrates (Figure 6). This may mean that at the current milk yield and feed intake, differences in effective $InsP_6$ degradation in the rumen are more likely to become relevant than in the past when rumen passage rate was lower.

DDGS

Sunflower meal

Rapeseed meal

Soybean meal

Soybeans

Faba beans

Barley

Wheat

Corn

30   40   50   60   70   80   90   100

ED_8 of $InsP_6$ (%)
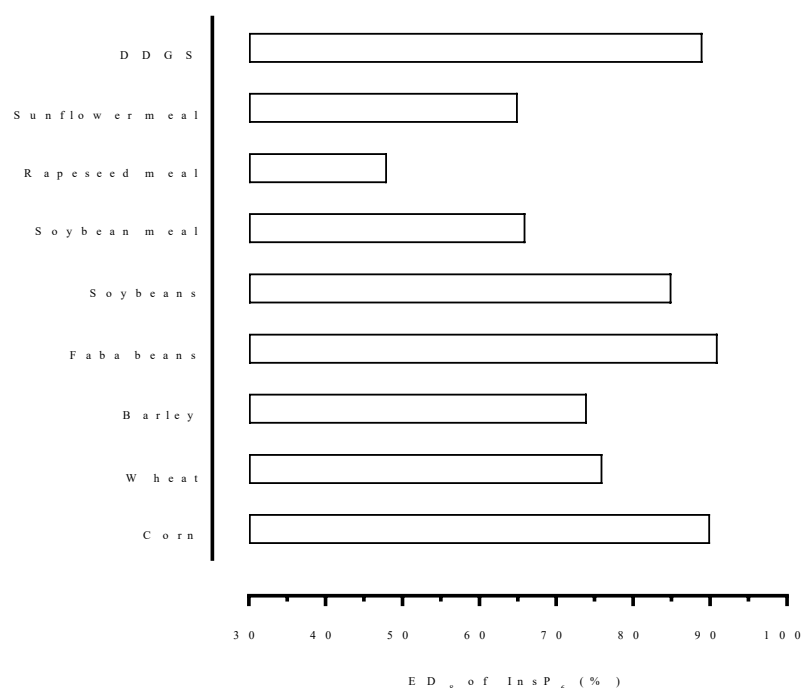
Figure 6. Effective degradation (ED) of $InsP_6$, predicted at a rumen passage rate of 8 %/h for different single concentrate feeds determined *in situ* (Haese et al., 2020)

Differences in P availability are poorly reflected in P requirement systems for cattle. For instance, the factorial requirement estimation in the German system assumes that mean P availability for cattle is 70 %, irrespective of the feed used (GfE, 2001). Such an estimate may not fully reflect the differences shown in Figure 5 and may call for a revision of the requirement estimates. However, from a pragmatic viewpoint, it can be questioned whether a deficit in P supply in the dairy industry is likely. Under conditions prevailing in the dairy industry in Europe and North America, oilseed meals are supplied because of their high content of rumen undegradable protein. Especially rapeseed meal, but also soybean meal and other oilseed meals, contain large amounts of P that are supplied together with the protein. The indication from a plethora of feeding trials is that no more than 4.0 g of total P/kg dry matter is required in the rations of high-yielding dairy cows, and the concentration can be lower for cows with lower milk yield. Among main forages, corn silage contains approximately 0.25 % P in dry matter, whereas the concentration is higher in grass or alfalfa products unless soils are deficient in P. Among concentrates, corn again belongs to those with a relatively low P concentration (approximately 0.3 % P in dry matter), whereas rapeseed meal contains approximately 1.3 % P in dry matter, and soybean meal approximately 0.7 %. Unless very specific combinations of low-P feedstuffs are used, typical rations of dairy cows contain P of plant origin in amounts exceeding their requirement. Therefore, I have long recommended that overall, mineral P does not need to be supplied in the rations for dairy cows. Mineral P might only be needed in exceptional cases when low-P feedstuffs, such as corn silage, corn, or sugar beet by-products are used in high amounts.

References

Ahmadi, H. and M. Rodehutscord. 2012. A meta-analysis of responses to dietary nonphytate phosphorus and phytase in laying hens. Poult. Sci. 91 2072-2078.

Ajuwon, K. M., V. Sommerfeld, V. Paul, M. Däuber, M. Schollenberger, I. Kühn, O. Adeola and M. Rodehutscord. 2020. Phytase dosing affects phytate degradation and Muc2 transporter gene expression in broiler starters. Poult. Sci. 99 981-991.

Applegate, T. J., R. Angel and H. L. Classen. 2003. Effect of dietary calcium, 25-hydroxycholecalciferol, or bird strain on small intestinal phytase activity in broiler chickens. Poult. Sci. 82 1140-1148.

Baumgärtel, T., B. U. Metzler, R. Mosenthin, R. Greiner and M. Rodehutscord. 2008. Precaecal and postileal metabolism of P, Ca and N in pigs as affected by different carbohydrate sources fed at low level of P intake. Arch. Anim. Nutr. 62 169-181.

Düngelhoef, M. and M. Rodehutscord. 1995. Wirkung von Phytasen auf die Verdaulichkeit des Phosphors beim Schwein. Übers. Tierernährg. 23 133-157.

Düngelhoef, M., M. Rodehutscord, H. Spiekers and E. Pfeffer. 1994. Effects of supplemental microbial phytase on availability of phosphorus contained in maize, wheat and triticale to pigs. Anim. Feed Sci. Techn. 49 1-10.

Faridi, A., A. Gitoee and J. France. 2015. A meta-analysis of the effects of nonphytate phosphorus on broiler performance and tibia ash concentration. Poult. Sci. 94 2753-2762.

GfE. 1994. Mitteilungen des Ausschusses für Bedarfsnormen der Gesellschaft für Ernährungsphysiologie: Die Bestimmung des verdaulichen Phosphors beim Schwein. Proc. Soc. Nutr. Physiol. 2 113-119.

GfE. 2001. Empfehlungen zur Energie- und Nährstoffversorgung der Milchkühe und Aufzuchtrinder. DLG-Verlag, Frankfurt am Main.

Gross, M. 2010. Fears over phosphorus supplies. Curr. Biol. 20 R386.

Haese, E., K. Müller, H. Steingass, M. Schollenberger and M. Rodehutscord. 2014. Effects of mineral and rapeseed phosphorus supplementation on phytate degradation in dairy cows. Arch. Anim. Nutr. 68 478-491.

Haese, E., J. Möhring, H. Steingass, M. Schollenberger and M. Rodehutscord. 2017a. Effect of dietary mineral phosphorus and phytate on in situ ruminal phytate disappearance from different concentrates in dairy cows. J. Dairy Sci. 100 3672-3684.

Haese, E., J. Krieg, G. Grubješić, A. Feyder and M. Rodehutscord. 2020. Determination of *in situ* ruminal degradation of phytate phosphorus from single and compound feeds in dairy cows using chemical analysis and near-infrared spectroscopy. Animal, online available, doi: 0.1017/S1751731120000221

Haese, E., M. Lengowski, E. Gräter, A. Föll, J. Möhring, H. Steingass, M. Schollenberger and M. Rodehutscord. 2017b. Ruminal phytate degradation of maize grain and rapeseed meal *in vitro* and as affected by phytate content in donor animal diets and inorganic phosphorus in the buffer. J. Anim. Physiol. Anim. Nutr. 101 868-880.

Hovenjürgen, M., M. Rodehutscord and E. Pfeffer. 1999. ME-Gehalt und Phosphor-Verdaulichkeit von schwach melassierten Trockenschnitzeln beim Schwein. Landb. Völkenr. Special issue 193 328-332.

Hovenjürgen, M., M. Rodehutscord and E. Pfeffer. 2003. Effect of fertilization and variety on digestibility of phosphorus from plant feedstuffs in pigs. J. Anim. Feed Sci. 12 83-93.

Hughes, A. L., J. P. Dahiya, C. L. Wyatt and H. L. Classen. 2009. Effect of Quantum phytase on nutrient digestibility and bone ash in White Leghorn laying hens fed corn-soybean meal-based diets. Poult. Sci. 88 1191-1198.

Ingelmann, C.-J., M. Witzig, J. Möhring, M. Schollenberger, I. Kühn and M. Rodehutscord. 2019. Phytate degradation and phosphorus digestibility in broilers and turkeys fed different corn sources with or without added phytase. Poult. Sci. 98 912-922.

Jing, M., S. Zhao, A. Rogiewicz, B. A. Slominski and J. D. House. 2018. Assessment of the minimal available phosphorus needs of laying hens: Implications for phosphorus management strategies. Poult. Sci. 97 2400-2410.

Jongbloed, A. W., Z. Mroz and P. A. Kemme. 1992. The effect of supplementary *Aspergillus niger* phytase in diets for pigs on concentration and apparent digestibility of dry matter, total phosphorus, and phytic acid in different sections of the alimentary tract. J. Anim. Sci. 70 1159-1168.

Kemme, P. A., U. Schlemmer, Z. Mroz and A. W. Jongbloed. 2006. Monitoring the stepwise phytate degradation in the upper gastrointestinal tract of pigs. J. Sci. Food Agric. 86 612-622.

Kemme, P. A., A. W. Jongbloed, Z. Mroz, J. Kogut and A. C. Beynen. 1999. Digestibility of nutrients in growing-finishing pigs is affected by *Aspergillus niger* phytase, phytate and lactic acid levels. 2. Apparent total tract digestibility of phosphorus, calcium and magnesium and ileal degradation of phytic acid. Livest. Prod. Sci. 58 119-127.

Khaksar, V., B. Meda and A. Narcy. 2017. Updating the available P requirements of broilers. In 21st European Symposium on Poultry Nutrition, Salou/Vila-seca, Spain, pp. 124-129.

Kincaid, R. L., D. K. Garikipati, T. D. Nennich and J. H. Harrison. 2005. Effect of grain source and exogenous phytase on phosphorus digestibility in dairy cows. J. Dairy Sci. 88 2893-2902.

Kluth, H., J. Gühne, K. Paditz and M. Rodehutscord. 2004. Untersuchungen zur Verdaulichkeit des Phosphors aus Erbsen beim Schwein. In VDLUFA-Kongress, Rostock, pp. 408-410.

Krause, G., M. Hovenjürgen, M. Rodehutscord and E. Pfeffer. 1997. Wirkung mikrobieller Phytase auf die Verdaulichkeit des Phosphors aus Corn-Cob-Mix beim Schwein. In VDLUFA-Kongress, Leipzig, pp. 147-150.

Leske, K. L. and C. N. Coon. 1999. A bioassay to determine the effect of phytase on phytate phosphorus hydrolysis and total phosphorus retention of feed ingredients as determined with broilers and laying hens. Poult. Sci. 78 1151-1157.

Létourneau-Montminy, M. P., A. Narcy, P. Lescoat, J. F. Bernier, M. Magnin, C. Pomar, Y. Nys, D. Sauvant and C. Jondreville. 2010. Meta-analysis of phosphorus utilisation by broilers

receiving corn-soyabean meal diets: influence of dietary calcium and microbial phytase. Animal 4 1844-1853.

Leytem, A. B., B. P. Willing and P. A. Thacker. 2008. Phytate utilization and phosphorus excretion by broiler chickens fed diets containing cereal grains varying in phytate and phytase content. Anim. Feed Sci. Techn. 146 160-168.

Mesina, V. G. R., L. V. Lagos, R. C. Sulabo, C. L. Walk and H. H. Stein. 2018. Effects of microbial phytase on mucin synthesis, gastric protein hydrolysis, and degradation of phytate along the gastrointestinal tract of growing pigs. J. Anim. Sci. 97 756-767.

Morse, D., H. H. Head and C. J. Wilcox. 1992. Disappearance of phosphorus in phytate from concentrates in vitro and from rations fed to lactating dairy cows. J. Dairy Sci. 75 1979-1986.

Neset, T.-S. S. and D. Cordell. 2012. Global phosphorus scarcity: identifying synergies for a sustainable future. J. Sci. Food Agric. 92 2-6.

Rapp, C., H.-J. Lantzsch and W. Drochner. 2001. Hydrolysis of phytic acid by intrinsic plant or supplemented microbial phytase (*Aspergillus niger*) in the stomach and small intestine of minipigs fitted with re-entrant cannulas. 3. Hydrolysis of phytic acid (IP_6) and occurence of hydrolysis products (IP_5, IP_4, IP_3 and IP_2). J. Anim. Physiol. Anim. Nutr. 85 420-430.

Ray, P. P., J. Jarrett and K. F. Knowlton. 2013. Effect of dietary phytate on phosphorus digestibility in dairy cows. J. Dairy Sci. 96 1156-1163.

Rodehutscord, M. 2006. Optimising the use of phosphorus sources in growing meat ducks. World's Poult. Sci. J. 62 513-523.

Rodehutscord, M. 2017. Advances in understanding the role of phytate in phosphorus and calcium nutrition of poultry. In Achieving sustainable production of poultry meat. Volume 2 Breeding and nutrition (ed. T. Applegate), pp. 165-180, Burleigh Dodds, Cambridge.

Rodehutscord, M., M. Faust and H. Lorenz. 1996. Digestibility of phosphorus contained in soybean meal, barley, and different varieties of wheat, without and with supplemental phytase fed to pigs and additivity of digestibility in a wheat-soybean-meal diet. J. Anim. Physiol. Anim. Nutr. 75 40-48.

Rodehutscord, M., M. Faust and C. Hof. 1997. Digestibility of phosphorus in protein-rich ingredients for pig diets. Arch. Anim. Nutr. 50 201-211.

Rodehutscord, M., M. Faust, M. Düngelhoef, H. Spiekers and E. Pfeffer. 1994. Zur Messung der Verdaulichkeit des Phosphors aus mineralischen Phosphor-Trägern sowie aus Mineralfuttern, Eiweißkonzentraten und Alleinfuttern für Schweine. J. Anim. Physiol. Anim. Nutr. 71 169-178.

Rodehutscord, M., H. Abel, W. Friedt, C. Wenk, G. Flachowsky, H.-J. Ahlgrimm, B. Johnke, R. Kühl and G. Breves. 2002. Consequences of the ban of by-products from terrestrial animals in livestock feeding in Germany and the European Union: alternatives, nutrient and energy cycles, plant production, and economic aspects. Arch. Anim. Nutr. 56 67-91.

Rosenfelder-Kuon, P., W. Siegert and M. Rodehutscord. 2020a. Effect of microbial phytase supplementation on P digestibility in pigs: a meta-analysis. Arch. Anim. Nutr. 74 1-18.

Rosenfelder-Kuon, P., N. Klein, B. Zegowitz, M. Schollenberger, I. Kühn, L. Thuringer, J. Seifert and M. Rodehutscord. 2020b. Phytate degradation cascade in pigs as affected by phytase supplementation and rapeseed cake inclusion in corn-soybean meal-based diets. J. Anim. Sci. online early available.

Rutherfurd, S. M., T. K. Chung and P. J. Moughan. 2014. Effect of microbial phytase on phytate P degradation and apparent digestibility of total P and Ca throughout the gastrointestinal tract of the growing pig. J. Anim. Sci. 92 189-197.

Sandberg, A.-S., T. Larsen and B. Sandström. 1993. High dietary calcium level decreases colonic phytate degradation in pigs fed a rapeseed diet. J. Nutr. 123 559-566.

Schemmer, R. 2020. Phosphorus digestibility and metabolizable energy concentrations of contemporary cereal grain varieties fed to growing pigs. Doctoral thesis, University of Bonn.

Schlemmer, U., K.-D. Jany, A. Berk, E. Schulz and G. Rechkemmer. 2001. Degradation of phytate in the gut of pigs - pathway of gastro-intestinal inositol phosphate hydrolysis and enzymes involved. Arch. Anim. Nutr. 55 255-280.

Shastak, Y., E. Zeller, M. Witzig, M. Schollenberger and M. Rodehutscord. 2014. Effects of the composition of the basal diet on the evaluation of mineral phosphorus sources and interactions with phytate hydrolysis in broilers. Poult. Sci. 93 2548-2559.

Siegert, W., T. Zuber, V. Sommerfeld, J. Krieg, D. Feuerstein, U. Kurrle and M. Rodehutscord. 2019. Prececal amino acid digestibility and phytate degradation in broiler chickens when using different oilseed meals, phytase and protease supplements in the feed. Poult. Sci. 98 5700-5713.

Sommerfeld, V., M. Schollenberger, I. Kühn and M. Rodehutscord. 2018a. Interactive effects of phosphorus, calcium, and phytase supplements on products of phytate degradation in the digestive tract of broiler chickens. Poult. Sci. 97 1177-1188.

Sommerfeld, V., S. Künzel, M. Schollenberger, I. Kühn and M. Rodehutscord. 2018b. Influence of phytase or *myo*-inositol supplements on performance and phytate degradation products in the crop, ileum, and blood of broiler chickens. Poult. Sci. 97 920-929.

Sommerfeld, V., A. G. Van Kessel, H. L. Classen, M. Schollenberger, I. Kühn and M. Rodehutscord. 2019. Phytate degradation in gnotobiotic broiler chickens and effects of dietary supplements of phosphorus, calcium, and phytase. Poult. Sci. 98 5562-5570.

Tamim, N. M. and R. Angel. 2003. Phytate phosphorus hydrolysis as influenced by dietary calcium and micro-mineral source in broiler diets. J. Agric. Food Chem. 51 4687-4693.

Tamim, N. M., R. Angel and M. Christman. 2004. Influence of dietary calcium and phytase on phytate phosphorus hydrolysis in broiler chickens. Poult. Sci. 83 1358-1367.

Taylor, A. E., M. R. Bedford, S. C. Pace and H. M. Miller. 2018. The effects of phytase and xylanase supplementation on performance and egg quality in laying hens. Br. Poult. Sci. 59 554-561.

Van der Klis, J. D., H. A. J. Versteegh, P. C. M. Simons and A. K. Kies. 1997. The efficacy of phytase in corn-soybean meal-based diets for laying hens. Poult. Sci. 76 1535-1542.

Yanke, L. J., H. D. Bae, L. B. Selinger and K.-J. Cheng. 1998. Phytase activity of anaerobic ruminal bacteria. Microbiology 144 1565-1573.

Zeller, E., M. Schollenberger, I. Kühn and M. Rodehutscord. 2015a. Hydrolysis of phytate and formation of inositol phosphate isomers without or with supplemented phytases in different segments of the digestive tract of broilers. J. Nutr. Sci. 4 e1.

Zeller, E., M. Schollenberger, M. Witzig, Y. Shastak, I. Kühn, L. E. Hoelzle and M. Rodehutscord. 2015b. Interactions between supplemented mineral phosphorus and phytase on phytate hydrolysis and inositol phosphates in the small intestine of broilers. Poult. Sci. 94 1018-1029.

Zeng, Z. K., D. Wang, X. S. Piao, P. F. Li, H. Y. Zhang, C. X. Shi and S. K. Yu. 2014. Effects of adding super dose phytase to the phosphorus-deficient diets of young pigs on growth performance, bone quality, minerals and amino acids digestibilities. Asian-Austr. J. Anim. Sci. 27 237-246.

From colostrum to weaning: nutritional regulation of gut function in the dairy calf

De la naissance au sevrage : régulation de la fonction intestinale chez le veau laitier par la nutrition

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Abstract

Raising healthy and productive calves is fundamental in ensuring the long-term success of the Canadian dairy industry. Unfortunately, pre-weaning calves suffer from the highest rates of morbidity (34%) and mortality (5%) amongst all animals on the dairy farm. Digestive disorders occurring before 2 weeks of age are the largest contributor to calf sickness and death; however, a multitude of previous research has demonstrated that proper nutritional management can have a positive impact on gut function and development, as well as productivity and health. During the first day of life, ensuring passive transfer in newborn calves is essential. Yet, colostrum contains an abundance of bioactive compounds aside from IgG, such as oligosaccharides, fatty acids and growth hormones, that may have beneficial effects on early life gut development. Maximizing pre-weaning whole milk or milk replacer (MR) intake is essential in promoting animal health and welfare, but there is growing interest in feeding elevated levels of MR as the macronutrient composition, namely lactose and fat, differs greatly from whole milk. Moreover, weaning calves from enhanced milk feeding programs can often result in health and production challenges and the source and level of starch in calf starter may further exacerbate this issue. Further research regarding optimal nutritional strategies during the newborn, pre-weaning and weaning stages is needed to allow industry representatives and dairy producers to make confident decisions to promote calf health, welfare and productivity.

Résumé

Le succès durable de l'industrie laitière canadienne repose sur l'élevage de veaux sains et productifs. Pourtant, les veaux non sevrés présentent les taux de morbidité (34 %) et de mortalité (5 %) les plus élevés parmi tous les groupes d'animaux d'une exploitation laitière. Les troubles digestifs survenant avant l'âge de deux semaines sont le principal facteur de maladie et de mortalité des veaux; cependant, une multitude de recherches ont montré qu'une bonne gestion de la nutrition peut avoir un effet positif sur la fonction et le développement de l'intestin, ainsi que sur la productivité et la santé. Pendant le premier jour de vie, il est essentiel d'assurer un transfert passif chez les veaux nouveau-nés. Heureusement, le colostrum contient une abondance de composés bioactifs autres que les IgG, tels que des oligosaccharides, des acides gras, de l'insuline et des hormones de croissance, qui peuvent avoir des effets bénéfiques sur le développement intestinal dans les premiers jours de vie. Il est indispensable de maximiser la consommation de lait entier ou de lactoremplaceurs avant le sevrage pour favoriser la santé et le bien-être des animaux, mais on constate un intérêt croissant pour l'alimentation riche en lactoremplaceurs en raison de leur composition en macronutriments, à savoir le lactose et les matières grasses, qui diffère grandement de celle du lait entier. Le sevrage des veaux alimentés avec des laits enrichis peut souvent entraîner des problèmes de santé et de production, et la source ainsi que la quantité d'amidon des aliments de démarrage pour les veaux peuvent encore aggraver ce problème. Des recherches supplémentaires concernant les stratégies nutritionnelles optimales pendant les phases de néonatalité, de pré-sevrage et de sevrage sont nécessaires pour permettre aux représentants de l'industrie et aux producteurs laitiers de prendre des décisions éclairées en faveur de la santé, du bien-être et de la productivité des veaux.

Introduction

The nutritional management of young dairy calves can have both short- and long-term effects, from influencing morbidity and mortality rates (Urie et al., 2018a) to reproductive efficiency and first-lactation milk yield (Faber et al., 2005; Soberon et al., 2012; Gelsinger et al., 2015). Although major improvements in calf nutritional strategies have been made over the past decade, dairy calves continue to suffer from the highest rates of mortality (5 - 6.4%) and morbidity (34%) amongst all animals on the dairy farm (Winder et al., 2018; Urie et al., 2018a). Digestive diseases and disorders (i.e. diarrhea) remain the most commonly reported cause of morbidity and mortality, accounting for over half of illnesses and one-third of deaths (Urie et al., 2018a). As such, digestive disorders represent a major cause of economic loss to dairy producers and cause concern from an animal welfare standpoint. Digestive disorders can often be mitigated through proper early life nutrition and health management regimens and developing strategies to improve calf gut health is fundamental in ensuring a profitable and sustainable dairy industry. Therefore, this review will focus on the effects of common nutritional strategies on gut development and function during three of the most challenging periods of the calf's life. Specifically, this paper will describe colostrum and transition milk feeding during the first days of life, plane of nutrition and diet composition during the first weeks of life, and differential weaning strategies and their effects on gut development.

Colostrum Feeding

Ensuring Passive Transfer

The calf is essentially born immune-deficient and relies on the feeding of 3-4 L of high quality (> 50 g of IgG/L) colostrum with a total bacterial count < 100,000 cfu/ml in the first hours of life (Weaver et al., 2000; McGuirk and Collins, 2004) to ensure its health and survival. Although this is well known, failure of passive transfer (**FPT;** serum IgG ≤ 10 mg/mL) still occurs in 12-19% of heifer calves (Beam et al., 2009; Shivley et al., 2009). High rates of FPT are associated with increased calf morbidity and mortality (Urie et al., 2018a) and decreased average daily gain (**ADG**; Robison et al., 1988). Moreover, feeding inadequate amounts of colostrum results in decreased survival and milk yield through the first (DeNise et al., 1989) and second lactation (Faber et al., 2005). Therefore, there is a significant need to improve colostrum management practices to not only promote calf health and welfare but also to ensure future productivity.

As previously mentioned, one of the most critical factors in ensuring the passive transfer of IgG is the timely feeding of colostrum after birth. Immediately after birth, the gut is considered "open" as the intestinal cells can non-selectively absorb large molecules, such as IgG, into circulation. Fischer et al. (2018a) demonstrated that calves fed colostrum at 6 and 12 h after birth displayed a 28% and 32% reduction in maximum serum IgG concentrations and the apparent efficiency of absorption of IgG, respectively, compared to calves fed immediately after birth. Importantly, calves fed at 6 and 12 h of life did not differ in any IgG parameters measured, suggesting that there may be a critical time point between 1 to 6 h of life in which the closure of the small intestine progresses to a finite degree. Unfortunately, the exact mechanism by which gut closure occurs has yet to be elucidated and future research is needed to determine the factors that control gut closure in the absence of colostrum feeding.

In addition to the quickness of colostrum feeding, Hare et al. (unpublished data) found that feeding multiple meals of colostrum can positively influence serum IgG concentrations. Specifically, calves fed colostrum for 3 d of life had greater maximum serum IgG concentrations compared to calves fed whole milk after the initial colostrum meal. Furthermore, calves fed multiple meals of colostrum had more persistent serum IgG concentrations (i.e. concentrations remained at a greater proportion of maximum IgG concentration reached) compared to calves only fed one meal, which may assist in preventing early life morbidity by reducing the high rates of digestive disorders. In addition, previous work found that the method by which colostrum is fed can influence serum IgG concentrations, with calves fed from a nipple bottle or an esophageal tube feeder having FPT rates of 14% and 9%, respectively (Shivley et al., 2018). However, a study directly investigating the influence of feeding 3 L of colostrum via nipple bottle or tube feeder on passive transfer (Desjardins-Morrissette et al., 2018) found no differences in serum IgG concentrations. The authors suggested that feeding large volumes of colostrum (≥ 3 L) via an esophageal tube feeder likely results in a negligible proportion of colostrum overflow into the rumen, which would not influence serum IgG concentrations to a large extent.

Establishing a Healthy Gut

Due to the importance of IgG in ensuring the health and survival of the newborn calf, colostrum has largely been known for promoting the acquisition of passive immunity over the past decades.

However, recent work has found that colostrum plays a key role in establishing a healthy gut bacterial community, which fundamentally promotes host health (Round and Mazmanian, 2009) and proper immune system development (Russell et al., 2013). In 2015, Malmuthuge et al. found that feeding colostrum resulted in a higher abundance of total bacteria and proportion of *Bifidobacterium* in the small intestine at 12 h of life, suggesting that colostrum is necessary for accelerating the microbial colonization of the gastrointestinal tract (**GIT**). Similarly, calves fed colostrum at 12 h of life tended to have a decreased proportion of *Bifidobacterium* and *Lactobacillus* associated with the colon mucosa compared to calves fed immediately after birth (Fischer et al., 2018a). It has been suggested that colostrum oligosaccharides (**OS**), which are up to 72 times greater in colostrum than in whole milk (Fischer-Tlustos et al., 2020), may mediate the early establishment of gut microbiota by acting as prebiotic compounds for beneficial bacterial species (Yu et al., 2013; Fischer et al., 2018b). Importantly, not feeding colostrum has been associated with an increased prevalence of small intestinal *Escherichia coli* (Malmuthuge et al., 2015) and delaying colostrum feeding until 12 h of life increases the abundance of opportunistic pathogens, namely *Enterococcus* and *Streptococcus* (Ma et al., 2019). Although these studies offer insight into the development of the calf gut microbiota and the potential influence that colostrum may have on promoting a healthy gut environment, how the colonization of these specific bacterial groups may affect future health and productivity needs to be explored further.

Colostrum not only contains compounds that promote gut bacterial colonization, but also contains high levels of nutrients, namely fat, that have been implicated in stimulating the secretion of glucagon-like peptide (**GLP**)-1 and GLP-2 (Burrin et al., 2003). Specifically, it was found that feeding colostrum by nipple bottle or tube-feeder both equally promoted the secretion of GLP-1 and GLP-2 (Desjardins-Morrissette et al., 2018), while delaying colostrum feeding until 12 h of life reduced plasma GLP-1 and GLP-2 concentrations compared to calves fed immediately after birth (Inabu et al., 2018). GLP-1 and GLP-2 are known for playing a key role in glucose homeostasis (Fukumori et al., 2012) and stimulating gastrointestinal growth (Taylor-Edwards et al., 2011), respectively, and thus it is clear that the early feeding of colostrum is important for ensuring optimal gut development and metabolism. In addition to promoting the secretion of gut hormones, fat also plays a key role in thermoregulation and certain omega fatty acids (**FA**) that are elevated in colostrum compared to whole milk (Hare et al., 2019) can have prolonged benefits in terms of antioxidant status and immune response (Opgenorth et al., 2019). In addition, colostrum contains elevated levels of insulin and insulin-like growth factor-1 (**IGF-1**; Blum and Hammon, 2000), both of which can promote intestinal cell proliferation, as well as antimicrobial compounds, such as lactoferrin and lactoperoxidase, which help to maintain a healthy gut environment (Pakkanen and Aalto, 1997). Therefore, although the multitude of bioactive colostral compounds have been largely overlooked during the past few decades, it is clear that colostrum has a much larger role in calf development than simply providing IgG.

Table 1. Levels of bioactive molecules in colostrum (milking 1) compared to whole milk[1] and their proposed functions.

Bioactive compound[2]	Colostrum	Whole Milk	Proposed Function
IgG, g/L	94.1	1.2	Passive immunity
Omega-3 FA, %	0.63	0.35	Immune modulation, antioxidant capacity
IGF-1, µg/L	310	<2	Intestinal development
Insulin, µg/L	65	1	Promote postnatal growth, gut development
3'SL, µg/mL	592.4	41.2	Prebiotic, immune modulation
Lactoferrin, g/L	1.84	ND	Local immunity, antimicrobial
Nucleotides, µmol/dL	258.7	15.6	Immune function, gastrointestinal development

[1]IgG, fat content, 3'SL concentration is reported in Fischer-Tlustos et al. (2020); Omega-3 FA % is reported in Hare et al. (2019); IGF-1, insulin and lactoferrin concentrations are reported in Blum and Hammon (2000); nucleotide concentrations are reported in Gill et al. (2011).
[2]FA = fatty acids; IGF-1 = insulin-like growth factor-1; 3'SL = 3'sialyllactose

Transition Milk

The aforementioned bioactive molecules are not only elevated in colostrum but are also present at high concentrations in transition milk (**TM**, milkings 2-6) compared to whole milk. For instance, TM contains elevated levels of primary acidic OS (Fischer-Tlustos et al., 2020), IGF-1, insulin (Blum and Hammon, 2000), nucleotides (Gill et al., 2011) and proportions of omega-3 FA (Hare et al., 2019) compared to whole milk. Recent research has also determined that feeding a 1:1 colostrum:whole milk mixture (**MIX**) to simulate TM feeding increased small intestinal surface area and cell proliferation in certain intestinal segments at 3 d of life (Pyo et al., 2020) and increased GLP-1 production (Inabu et al., 2019), which can have beneficial effects on energy use. Furthermore, feeding MIX improved IgG persistency (91% of maximum concentration, Cmax) compared to calves fed whole milk (75% of Cmax) after the initial colostrum feeding (Hare et al., unpublished data). Although IgG in MIX, or TM, will not be transported circulation after 24 h of life (Weaver et al., 2000), antibodies remaining in the lumen may assist in providing local immunity against enteric pathogens (Berge et al., 2009). Despite this knowledge, a large proportion of dairy producers continue to discard TM and transition calves directly to milk replacer (**MR**) or whole milk after the first colostrum feeding, which may result in missed opportunities to improve newborn calf gut health and metabolism. The current lack of information regarding TM feeding emphasizes the need for future research describing the importance of these specific compounds on metabolic regulation and development in the newborn calf, which may lead the development of TM or colostrum:whole milk feeding strategies to improve calf gut health.

Milk Nutrition

Plane of Nutrition

After colostrum or TM feeding for the first days of life, calves are often transitioned to elevated (≥ 8 L or 1.2 kg of MR powder/d, 67% of Canadian producers) or conventional (≤ 6 L or 900 kg MR powder/d, 33% of Canadian producers) planes of milk nutrition (Winder et al., 2018). Conventional programs aim to encourage early starter intake to facilitate rumen development (Tamate et al., 1962), which may result in less susceptibility to health and production challenges during weaning. However, calves fed elevated planes of nutrition have improved animal welfare through reduced starvation-associated behaviours (De Paula Vieira et al., 2008) and increased potential to produce more milk during lactation, improved mammary development, reduced age at first calving (Soberon et al., 2012) and improved immune function (Ballou et al., 2015) compared to calves fed conventional levels of milk. A recent study by Haisan et al. (2019) demonstrated that all calves offered large volumes of milk were able to consume over 8 L of whole milk/d and up to 10 L/d using an automated feeder during the first week of life. Although this clearly demonstrates that elevated feeding programs are synergistic with the calf's natural ability to consume large volumes of milk during early life, the majority of Canadian producers still limit calves to 4 L of milk/d from days 1-7 of life (Vasseur et al., 2010). At this time, all metabolizable nutrients are consumed directly from milk due to negligible starter intake and maintenance requirements alone equal ~3 L of milk/d; therefore, feeding only 4 L largely restricts energy for growth. This large restriction in metabolizable energy is likely why calves in the aforementioned study only gained 400 g/d during the first week of life when limit-fed 5 L/d compared to calves fed over 8 L/d, who gained up to 800 g/d (Haisan et al., 2019).

Despite the well-known benefits of feeding elevated planes of nutrition, many producers do not adopt this practice as they perceive it as unfeasible without automated feeding. This is mainly due to concerns around feeding large volumes of milk in 2 meals/d, because previous research suggested that feeding a large volume of milk in a single feeding can lead to abomasal overflow of milk into the rumen (Berends et al., 2015) and reduced insulin sensitivity (Bach et al., 2013). However, a 2016 study (Ellingsen et al.) demonstrated that calves can consume 5-7 L of milk per meal without any overflow into the rumen. Furthermore, MacPherson et al. (2018) found that calves fed 8 L of milk over 2 or 4 meals/d did not display any differences in insulin sensitivity during a glucose tolerance test. Calves fed only 2 meals per day had a decreased rate of abomasal emptying, indicating that glucose delivery was slowed, which may have regulated insulin response. It is important to note that calves were fed 8 L/d over 2 meals beginning from the first week of life, and this may be a crucial metabolic developmental window in which the calf adapts to consuming high volumes of milk; however, further research regarding the long-term effects of this practice on calf metabolism and development is warranted.

Milk Replacer vs. Whole Milk Composition

In Canada, 50% of dairy producers feed calves milk replacer (Vasseur et al., 2010) as opposed to whole milk. Producers generally adopt MR feeding because the nutrition provided is consistent, and it is clean and convenient. However, previous work has shown advantages to feeding whole milk compared to MR, including lower mortality and morbidity rates (Godden et al., 2005), higher energy content and a balanced supply of nutrients (Davis and Drackley, 1998). There is growing interest in the macronutrient composition of MR as it contains more lactose (45 vs.

35%) and less fat (18 vs. 30%) compared to whole milk. Still, little information exists regarding how feeding MR - especially large volumes - may affect calf gut health. High fat consumption is essential in meeting the energy demands of the young calf and increased inclusion in liquid feed can reduce the odds of mortality by 3-fold in preweaned calves (Urie et al., 2018a). Furthermore, MR is typically formulated using animal- and plant-based fats that often differ greatly in FA profiles compared to whole milk, which can negatively affect calf productivity and health (Jenkins et al., 1985). Recent work by Welboren et al. (2019a) demonstrated that feeding MR with high fat and low lactose content during the first week of life tended to delay abomasal emptying compared to calves fed a traditional MR. This may be beneficial in delaying the digestion of nutrients to allow for better absorption and may have provided reasoning for calves fed a high fat MR experiencing a lesser rise in glucose and insulin concentrations as opposed to calves fed a traditional MR (Welboren et al., 2019a). This finding suggests that high lactose inclusion may negatively affect glucose homeostasis; however, whether this practice leads to the development of insulin resistance requires further investigation. In addition, high lactose inclusion in MR can drastically increase osmolality (~400-600 mOsm/L) compared to whole milk (300 mOsm/L), which can increase intestinal permeability and potentially disturb gut mucosal structure and function (Wilms et al., 2019). However, current research on this topic is lacking and/or conflicting (Welboren et al., 2019b), and it is clear that more research is required to identify calf metabolic and intestinal development responses as calves are progressively fed larger volumes of MR.

Weaning Transition

In nature, calves would consume > 10 L of milk/d over multiple small meals, with weaning occurring at 7-14 months of age (Reinhardt and Reinhardt, 1982). This is a stark contrast to the majority of weaning strategies in the dairy industry, in which weaning typically occurs around 9 weeks of age (Urie et al., 2018b). In an effort to limit feeding costs, encourage early starter intake and rumen development, many operations wean calves at 4-6 weeks of life. Unfortunately, the digestive tract of calves fed elevated planes of nutrition during the pre-weaning period are not equipped to digest large amounts of solid feed during the weaning and post-weaning periods. This often results in production and health challenges, likely due to the decreased digestibility of fiber, NDF, and gross energy (Terre et al., 2007; Hill et al., 2016; Dennis et al., 2018). Research has shown that the production challenges experienced during the weaning transition in calves fed elevated planes of milk pre-weaning can be improved by extending weaning from 6 to 8 weeks of life (Eckert et al., 2015; Meale et al., 2015). In addition, potential negative outcomes can be further mitigated by utilizing the "step-down" weaning method when weaning from elevated planes of milk. Specifically, research by Khan et al. (2007a,b) found that calves receiving elevated levels of milk at 20% of BW until d 23, followed by a step-down to 10% of BW from d 23 to d 44 had increased solid feed intake and weight gain compared to calves fed at 10% of BW until d 44. As such, this may be a feasible and efficient strategy to maximize weight gain while simultaneously achieving early weaning and rumen development. Moreover, the increasing implementation of automated feeding will likely play a large role in optimizing weaning strategies in the future. Automated feeding can be used to achieve linear declines in milk intake, which can result in increased performance compared to calves reduced at abrupt 2 L intervals (Welboren et al., 2019c), as well as to design individualized strategies based on starter intake.

The GIT undergoes significant changes during weaning, with the total volume of the rumen increasing from 30% to 70% of the entire forestomach (Warner et al., 1965) and short chain fatty acids (SCFA) accounting for 80% of the calf's energy after weaning. The rumen transcriptome and microbiome also undergo rapid maturation, with increased expression of gut barrier (Malmuthuge et al., 2013) and metabolic genes (Connor et al., 2013) and substrates in calf starter shifting microbial populations (Meale et al., 2017). Calves are often fed high starch (> 30%) in calf starter in an effort to initiate rapid rumen development; however, this can result in ruminal acidosis due to the accumulation of SCFA. Recent work has found that it can take up to 5 weeks after weaning for the rumen environment of calves fed elevated planes of nutrition pre-weaning to be in a state that is not considered ruminal acidosis (Van Niekerk et al., unpublished data). Furthermore, severe acidosis may also affect the hindgut (Li et al., 2012), as evidenced by high levels of fecal starch in calves fed elevated planes of nutrition pre-weaning (Eckert et al., 2015; Van Niekerk et al., 2020). The site of fermentation may be shifted depending on the source of starch, with calves fed elevated levels of milk and whole corn in calf starter displaying decreased fecal pH for 2 weeks following weaning compared with calves fed flaked corn (Van Niekerk et al., 2020). These results suggest that whole corn may shift the site of fermentation to the lower gut, possibly resulting in hindgut acidosis. This is highly unfavourable, as it can lead to systemic inflammatory responses that negatively affect both calf production and health. At present, few studies have characterized the functional changes occurring in the lower gut during weaning. Further research regarding the combined impacts of pre-weaning planes of nutrition, source and level of starch in starter, and weaning strategy is needed.

Conclusion

It is clear that nutritional management during the newborn, pre-weaning and weaning phases can largely impact growth performance, health and gut function and development. Although colostrum research has largely focused on improving passive transfer over the past few decades, colostrum and transition milk contain a multitude of bioactive molecules beyond IgG that can positively influence gastric development and metabolism. Furthermore, maximizing nutrient intake from milk or MR during early life is essential in supporting growth when starter intake is negligible; however, a large knowledge gap currently exists around how current MR formulations may affect calf gut development and metabolism when elevated planes of nutrition are fed. Weaning calves from elevated planes of nutrition can result in health and production challenges, which can be mitigated through the use of step-down weaning methods and may be further improved and individualized with the recent and rapid implementation of automated feeding. In conclusion, further research is needed to determine the functional changes and long-term consequences of differing nutritional strategies during the first days, weeks and months of the calf's life in order to maximize calf health and productivity.

References

Bach, A., L. Domingo, C. Montoro, and M. Terré. 2013. Short communication: insulin responsiveness is affected by the level of milk replacer offered to young calves. J. Dairy Sci. 96:4634–4637.

Ballou, M.A., D.L. Hanson, C.J. Cobb, B.S. Obeidat, M.D. Sellers, A.R. Pepper-Yowell, J.A. Carroll, T.J. Earleywine, and S.D. Lawhon. 2015. Plane of nutrition influences the performance, innate leukocyte responses and resistance to an oral Salmonella enterica serotype Typhimurium challenge in Jersey calves. J. Dairy Sci. 98(3):1972-1982.

Beam, A.L., J.E. Lombard, C.A. Kopral, L.P. Garber, A.L. Winter, J.A. Hicks, and J.L. Schlater. 2009. Prevalence of failure of passive transfer of immunity in newborn heifer calves and associated management practices on US dairy operations. J. Dairy Sci. 92:3973-3980.

Berends, H., J. J. van den Borne, N. Stockhofe-Zurwieden, M. S. Gilbert, T. Zandstra, W. F. Pellikaan, C. G. van Reenen, E. A. M. Bokkers, and W. J. J. Gerrits. 2015. Effects of solid feed level and roughage-to-concentrate ratio on ruminal drinking and passage kinetics of milk replacer, concentrates, and roughage in veal calves. J. Dairy Sci. 98:5621–5629.

Berge, A.C.B., T.E. Besser, D.A. Moore, and W.M. Sisco. 2009. Evaluation of the effects of oral colostrum supplementation during the first fourteen days on the health and performance of pre-weaned calves. J. Dairy Sci. 92:286-295.

Blum, J.W. and H. Hammon. 2000. Colostrum effects on the gastrointestinal tract, and on nutritional, endocrine and metabolic parameters in neonatal calves. Live. Prod. Sci. 66:151-159.

Burrin, D.G., B. Stoll, and X. Guan. 2003. Glucagon-like peptide-2 function in domestic animals. Domest. Anim. Endocrinol. 24:103-122.

Connor, E. E., R. L. Baldwin, C. Li, R. W. Li, and H. Chung. 2013. Gene expression in bovine rumen epithelium during weaning identifies molecular regulators of rumen development and growth. Funct. Integr. Genomics.13:133–142.

Davis, C.L. and J.K. Drackley. 1998. The development, nutrition, and management of the young calf. Iowa State Univ. Press, Ames.

DeNise, S.K., J.D. Robison, G.H. Stott, and D.V. Armstrong. 1989. Effects of passive immunity on subsequent production in dairy heifers. J. Dairy Sci. 72:552–554.

Dennis, T.S., F.X. Suarez-Mena, T.M. Hill, J.D. Quigley, R.L. Schlotterbeck, R.N. Klopp, G.J. Lascano, and L. Hulbert. 2018. Effects of gradual and later weaning ages when feeding high milk replacer rates on growth, textured starter digestibility, and behavior in Holstein calves from 0 to 4 months of age. Journal of dairy science. 101(11):9863-75.

de Paula Vieira, A., Guesdon, V., de Passillé, A.M., von Keyserlingk, M.A.G., and Weary, D.M. 2008. Behavioural indicators of hunger in dairy calves. Appl. Anim. Behav. Sci. 109: 180–189.

Desjardins-Morrissette, M., J.K. van Niekerk, D. Haines, T. Sugino, M. Oba, and M.A. Steele. 2018. The effect of tube vs. bottle feeding colostrum on IgG absorption, abomasal emptying and plasma hormone concentrations in newborn calves. J. Dairy Sci. 101:4168–4179.

Eckert, E., H. E. Brown, K. E. Leslie, T. J. DeVries, and M. A. Steele. 2015. Weaning age affects growth, feed intake, gastrointestinal development, and behaviour in Holstein calves fed an elevated plane of nutrition during the preening stage. J. Dairy Sci. 98:6315-6326.

Ellingsen, K., C.M. Mejdell, N. Ottesen, S. Larsen, and A.M. Grondahl. 2016. The effect of large milk meals on digestive physiology and behaviour in dairy calves. Physiol. Behav. 154:169–174.

Faber, S.N., N.E. Faber, T.C. McCauley, and R.L. Ax. 2005. Effects of colostrum ingestion on lactational performance. Prof. Anim. Sci. 21:420-425.

Fischer, A.J., Y. Song, Z. He, D.M. Haines, L.L Guan, and M.A. Steele. 2018a. Effect of delaying colostrum feeding on passive transfer and intestinal bacterial colonization in neonatal male Holstein calves. J. Dairy Sci. 101:3099-3109. https://doi.org/ 10.3168/jds.2017-13397

Fischer, A. J., N. Malmuthuge, L. L. Guan, and M. A. Steele. 2018b. Short communication: The effect of heat treatment of bovine colostrum on the concentration of oligosaccharides in colostrum and in the intestine of neonatal male Holstein calves. J. Dairy Sci. 101:401-407.

Fischer-Tlustos, A.J., K. Hertogs, J.K. Van Niekerk, M. Nagorske, D.M. Haines, and M.A. Steele. 2020. Oligosaccharide concentrations in colostrum, transition milk, and mature milk of primi- and multi-parous Holstein cows during the first week of lactation. J. Dairy Sci. In Press: Accepted Dec 6, 2019.

Fukumori, R. T., T. Mita, T. Sugino, T. Obitsu, and K. Taniguchi. 2012. Plasma concentrations and effects of glucagon-like peptide-1 (7-36) amide in calves before and after weaning. Domest. Anim. Endocrinol. 43:299-306.

Gelsinger, S.L., Heinrichs, A.J., and Jones, C.M. 2016. A metaanalysis of the effects of preweaned calf nutrition and growth on first-lactation performance. J. Dairy Sci. 99(8):6206–6214.

Gill, B.D., H.E. Indyk, and M. Manley-Harris. 2011. Determination of total potentially available nucleosides in bovine milk. Int. Dairy J. 21:34–41.

Godden, S.M., J.P. Fetrow, J.M. Freitag, L.R. Green, and S.J. Wells. 2005. Economic analysis of feeding pasteruized non-salable milk versus conventional milk replacer to dairy calves. J. Am. Vet. Med. Assoc. 226:1547-1554.

Haisan, J., M.A. Steele, D.J. Ambrose, and M. Oba. 2019. Effects of amount of milk fed, and starter intake, on performance of group-housed dairy heifers during the weaning transition. Appl. Anim. Sci. 35:88–93.

Hare, K.S., K. Hertogs, A. Fischer, P. Vahmani, M.E.R. Dugan, and M. Steele. 2019. Omega-3 and -6 fatty acids are more abundant in colostrum than transition and whole milk. J. Dairy Sci. 102(Suppl. 1):154.

Hill, T.M., J.D. Quigley, H.G. Bateman II, F.X. Suarez-Mena, T.S. Dennis, and R.L. Schlotterbeck. 2016. Effect of milk replacer program on calf performance and digestion of nutrients in dairy calves to 4 months of age. J. Dairy Sci. 99(10):8103-10.

Inabu, Y., A. Fischer, Y. Song, L.L. Guan, M. Oba, M.A. Steele, and T. Sugino. 2018. Short communication: The effect of delayed colostrum feeding on plasma concentrations of glucagon-like peptide 1 and 2 in newborn calves. J. Dairy Sci. 101:6627-6631.

Inabu, Y., J. Pyo, S. Pletts, L.L. Guan, M.A. Steele, and T. Sugino. 2019. Effect of extended colostrum feeding on plasma glucagon-like peptide-1 concentration in newborn calves. J. Dairy Sci. 102:4619–4627.

Jenkins, K.J., J.K.G. Kramer, F.D. Sauer, and D.B. Emmons. 1985. Influence of triglycerides and free fatty acids in milk replacers on calf performance, blood plasma, and adipose lipids. J. Dairy Sci. 68:669–680.

Khan, M. A., H. J. Lee, W. S. Lee, H. S. Kim, S. B. Kim, K. S. Ki, J. K. Ha, H. G. Lee, and Y. J. Choi. 2007a. Pre- and postweaning performance of Holstein female calves fed milk through step-down and conventional methods. J. Dairy Sci. 90:876–885.

Khan, M. A., H. J. Lee, W. S. Lee, H. S. Kim, K. S. Ki, T. Y. Hur, G. H. Suh, S. J. Kang, and Y. J. Choi. 2007b. Structural growth, rumen development, and metabolic and immune responses of Holstein male calves fed milk through step-down and conventional methods. J. Dairy Sci. 90:3376–3387.

Li, R.W., E.E. Connor, C. Li, R.L. Baldwin VI, and M.E. Sparks. 2012. Characterization of the rumen microbiota of pre-ruminant calves using metagenomics tools. Environ. Microbiol. 14:129-139.

Ma, T., E. O'Hara, Y. Song, A. Fischer, Z. He, M.A. Steele, and L.L. Guan. 2019. Altered mucosa-associated microbiota in the ileum and colon of neonatal calves in response to delayed first colostrum feeding. J. Dairy Sci. 102:7073-7086.

MacPherson, J., S.J. Meale, K. Macmillan, J. Haisan, C.J. Bench, M. Oba, and M.A. Steele. 2018. Effects of feeding frequency of an elevated plane of milk replacer and calf age on behavior, and glucose and insulin kinetics in male Holstein calves. Animal. 13:1385–1393.

Malmuthuge, N., M. Li, L. A. Goonewardene, M. Oba, and L. L. Guan. 2013. Effect of calf starter feeding on gut microbial diversity and expression of genes involved in host immune responses and tight junctions in dairy calves during weaning transition. J. Dairy Sci. 96:189-200.

Malmuthuge, N., Y. Chen, G. Liang, L.A. Goonewardene, and L.L. Guan. 2015. Heat-treated colostrum feeding promotes beneficial bacteria colonization in the small intestine of neonatal calves. J. Dairy Sci. 98:8044-8053.

McGuirk, S.M. and M. Collins. 2004. Managing the production, storage and delivery of colostrum. Vet. Clin. North Am. Food Anim. Pract. 20:593-603.

Meale, S. J., L. N. Leal, J. Martín-Tereso, and M. A. Steele. 2015. Delayed weaning of Holstein bull calves fed an elevated plane of nutrition impacts feed intake, growth and potential markers of gastrointestinal development. Anim. Feed Sci. Tech. 209:268–273.

Meale, S. J., S. C. Li, P. Azevedo, H. Derakhshani, T. J. DeVries, J. C. Plaizier, M. A. Steele, and E. Khafipour. 2017. Weaning age influences the severity of gastrointestinal microbiome shifts in dairy calves. Sci. Rep. 7: doi:10.1038/s41598-017-00223-7.

Opgenorth, J., L.M. Sordillo, and M.J. VandeHaar. 2019. Colostrum supplementation with omega-3 fatty acids and α-tocopherol decreases indicators of oxidative stress and alters plasma fatty acid profile in newborn calves during the first week of life. J. Dairy Sci. 102(Suppl. 1):86.

Pakkanen, R. and J. Aalto. 1997. Growth factors and antimicrobial factors of bovine colostrum. Int. Dairy J. 7(5):285-297.

Pyo, J., K. Hare, S. Pletts, Y. Inabu, D. Haines, T. Sugino, L. L. Guan, and M. Steele. 2020. Feeding colostrum or a 1:1 colostrum:milk mixture for 3 d postnatal increase small intestinal development and minimally influences plasma GLP-2 and serum IGF-1 concentrations in Holstein bull calves. J. Dairy Sci. In Press: Accepted 01/04/2020.

Reinhardt, V. and A. Reinhardt. 1981. Natural sucking performance and age at weaning in zebu cattle (Bos indicus). J. Agric. Sci. 96:309–312.

Robison, J.D., G.H. Stott, and S.K. DeNise. 1988. Effects of passive immunity on growth and survival in the dairy heifer. J. Dairy Sci. 71:1283-1287.

Round, J.L. and S.K. Mazmanian. 2009. The gut microbiota shapes intestinal immune responses during health and disease. Nat. Rev. Immunol. 9:313-323.

Russel, S.L., M.J. Gold, B.P. Willing, L. Thorson, K.M. McNagny, and B.B. Finlay. 2013. Perinatal antibiotic treatment affects murine microbiota, immune responses and allergic asthma. Gut Microbes. 4:158-164.

Shivley, C.B., J.E. Lombard, N.J. Urie, D.M. Haines, R. Sargent, C.A. Kopral, T.J. Earleywine, J.D. Olson, and F.B. Garry. 2018. Preweaned heifer management on US dairy operations: Part II. Factors associated with colostrum quality and passive transfer status of dairy heifer calves. J. Dairy Sci. 101:9185-9198.

Soberon, F., E. Raffrenatio, R.W. Everett, and M.E. van Amburgh. 2012. Preweaning milk replacer intake and effects on long-term productivity of dairy calves. J. Dairy Sci. 95:783-793.

Tamate, H., R. Getty, A. D. McGilliard, and N. L. Jacobson. 1962. Effect of various dietaries on anatomical development of stomach in calf. J. Dairy Sci. 45:408-420.

Taylor-Edwards, C.C., D.G. Burrin, J.J. Holst, K.R. McLeod, and D.L. Harmon. 2011. Glucagon-like peptide-2 (GLP-2) increases small intestinal blood flow and mucosal growth in ruminating calves. J. Dairy Sci. 94:888-898.

Terré, M., M. Devant, and A. Bach. 2007. Effect of level of milk replacer fed to Holstein calves on performance during the preweaning period and starter digestibility at weaning. Livest. Sci. 110:82–88.

Urie, N.J., J.E. Lombard, C.B. Shivley, C.A. Kopral, A.E. Adams, T.J. Earleywine, J.D. Olson, and F.B. Garry. 2018a. Preweaned heifer management on US dairy operations: Part V. Factors associated with morbidity and mortality in preweaned dairy heifer calves. J. Dairy Sci. 101:9229–9244.

Urie, N.J., J.E. Lombard, C.B. Shivley, C.A. Kopral, A.E. Adams, T.J. Earleywine, J.D. Olson, and F.B. Garry. 2018b. Preweaned heifer management on US dairy operations: Part I. Descriptive characteristics of preweaned heifer raising practices. J. Dairy Sci. 101:9168-9184.

Van Niekerk, J.K., A.J. Fischer-Tlustos, L.L. Diekun, J.D. Quigley, T. Dennis, X. Suarez-Mena, T.M. Hill, R. Schlotterbeck, L.L. Guan, and M.A. Steele. 2020. Impact of amount of milk replacer fed and the processing of corn in starter on growth performance, nutrient digestibility and rumen and fecal fibrolytic bacteria of dairy calves. J. Dairy Sci. 103(3):2186-2199.

Vasseur, E., F. Borderas, R.I. Cue, D. Lefebvre, D. Pellerin, J. Rushen, K.M. Wade, and A.M. de Passille. 2010. A survey of dairy calf management practices in Canada that affect animal welfare. J. Dairy Sci. 93:1307–1315.

Warner, R. G., W. P. Flatt, and J. K. Loosli. 1956. Dietary factors influencing the development of the ruminant stomach. Agric. Food Chem. 4:788–801.

Weaver, D.M., J.W. Tyler, D.C. VanMetrem, D.E. Hostetler, and G.M. Barrington. 2000. Passive transfer of colostral immunoglobulins in calves. J. Vet. Intern. Med. 14:569-577.

Welboren, A., B. Hatew-Chuko, L. Leal, J. Martín-Tereso, and M. Steele. 2019a. Effects of a high lactose milk replacer on glucose metabolism in neonatal calves. WCDS Proc. 31:236.

Welboren, A.C., B. Hatew, L. Leal, J. Martin-Tereso, and M.A. Steele. 2019b. 80 Effects of macronutrient composition of milk replacer on body composition and intestinal development in neonatal dairy calves. J. Anim. Sci. 97(Suppl. 3):70–71.

Welboren, A.C., L.N. Leal, M.A. Steele, M.A. Khan, and J. Martín-Tereso. 2019c. Performance of ad libitum fed dairy calves weaned using fixed and individual methods. Animal. 1-8.

Wilms, J., H. Berends, and J. Martin-Tereso. 2019. Hypertonic milk replacers increase gastrointestinal permeability in healthy dairy calves. J. Dairy Sci. 102:1237–1246.

Winder, C.B., C.A. Bauman, T.F. Duffield, H.W. Barkema, G.P. Keefe, J. Dubuc, F. Uehlinger, D.F. Kelton. 2018. Canadian National Dairy Study: Heifer calf management. J. Dairy Sci. 101:10565-10579.

Yu, Z-T., C. Chen, and D.S. Newburg. 2013. Utilization of major fucosylated and sialylated human milk oligosaccharides by isolated human gut microbes. Glycobiology. 23(11):1281-1292.

Influencing microbiota-host interactions by dietary strategies to improve intestinal health

Influencer les interactions microbiote-hôte pour améliorer la santé intestinale par des stratégies alimentaires

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Abstract

The intestinal tract of monogastrics and ruminants is a key organ in health and disease, and optimal intestinal health is essential for animal performance. While the intestine is crucial for digestion of feed components, it has become clear that this organ is also involved in cross-organ communication and extraintestinal disease. The intestinal microbiota plays an important role in digestion and host health, because of the breakdown of feed components and the production of a large panel of metabolites, that can have a signaling function to other microbiota, but also to host cells. Because epithelial cells are the first host cells that sense these microbial metabolites, these are major drivers for transmitting bacterial signals to the host, and as such mucosa-associated microbial populations are main drivers of intestinal health. These microbial populations can either be (opportunistic) pathogenic, such as *E. coli* or *C. perfringens*, or can consist of specific anaerobic genera that colonize the mucus layer and produce beneficial metabolites, such as butyrate. Novel insights have been generated on the function of specific metabolites on intestinal inflammation, epithelial cell proliferation and differentiation, and on effects on general physiological responses of the animal. This has led to novel methods of designing nutritional strategies to maintain health. In addition, the effect of classical feed additives, such as probiotics, prebiotics and the more recent essential oils, can at least partly be explained by microbial metabolic shifts in the gut, although direct effects on host cells also have their role. Interestingly, the concepts of microbe-host interactions to maintain optimal host health seem to have some universal characteristics, that are independent of the animal host. The current paper will describe the modulation of microbial composition to produce metabolites that are increasing resilience of animals towards infectious and non-infectious challenges that can compromise health.

Résumé

Le tractus intestinal des monogastriques et des ruminants est un organe déterminant pour leur santé, et la performance des animaux repose en bonne partie sur leur aptitude à maintenir une santé intestinale optimale. Si l'intestin est indispensable pour la digestion des composants alimentaires, il est maintenant reconnu que cette structure intervient aussi sur le plan de la communication entre les organes et sur celui des maladies extra-intestinales. Le microbiote intestinal joue un rôle important dans la digestion et la santé chez l'hôte, parce qu'il participe à la dégradation des composants alimentaires et à la production d'un large éventail de métabolites qui peuvent avoir une fonction de signalisation pour d'autres microbiotes, mais aussi pour des cellules hôtes. Les cellules épithéliales étant les premières cellules hôtes à détecter ces métabolites microbiens, elles sont les principaux moteurs de la transmission des signaux bactériens à l'hôte et, à ce titre, les populations microbiennes associées aux muqueuses comptent parmi les principaux acteurs de la santé intestinale. Ces populations microbiennes peuvent être soit pathogènes (opportunistes), comme *E. coli* ou *C. perfringens*, soit composées de genres anaérobies spécifiques qui colonisent la couche de mucus et produisent des métabolites bénéfiques, comme le butyrate. Nous en savons maintenant davantage sur la fonction de certains métabolites dans le processus inflammatoire intestinal et leurs effets sur la prolifération et la différenciation des cellules épithéliales ainsi que sur les réponses physiologiques générales chez l'animal. Cela a amené à revoir les façons de concevoir les stratégies nutritionnelles destinées à préserver la santé. En outre, l'action des additifs alimentaires classiques, tels que les probiotiques, les prébiotiques et, plus récemment, les huiles essentielles, peut s'expliquer au moins en partie par des modifications du métabolisme microbien dans les intestins, bien que les effets directs sur les cellules hôtes fassent également partie de l'équation. Il est intéressant de noter que les concepts d'interactions microbe-hôte pour maintenir la santé optimale de l'hôte semblent présenter certaines caractéristiques universelles qui sont indépendantes de l'animal hôte. Le présent article décrit la modification du microbiote visant à produire des métabolites qui augmentent la résilience des animaux face aux agressions infectieuses et non infectieuses qui peuvent compromettre la santé.

Introduction

Animals used for food production have been genetically selected for feed intake and muscle development (or milk and egg production) and are therefore divergent from their ancestors. In addition, these animals are reared in conditions that favor fast spread of pathogens. The high uptake of feed and the fast growth make these animals prone to intestinal disorders, what has been neglected in the past because of the use of low doses of specific antimicrobial compounds, called antimicrobial growth promoters (AGPs). These were used worldwide to maintain the profitability of the broiler and pig industry. These antibiotic substances, added at sub-therapeutic level as feed additives, increased animal performance. A ban on the use of AGPs, mainly driven by consumer concerns about increases in antimicrobial resistance, was installed in the EU in 2006, followed by a global concern that led to a decreased use or ban, depending on the region. In 2012 the Center for Veterinary Medicine of the US Food and Drug Administration wrote a 'Guidance for Industry' document mentioning that antibiotics should only be used in case of specific diseases and not for growth promotion. The mode of action of the AGPs is still under

debate, but a variety of mechanisms have been proposed, including a reduction in total bacterial counts in the gut (and consequently less competition for nutrients), a reduction of specific pathogens (e.g. *Clostridium perfringens*), a decreased abundance of specific harmful bacterial properties (e.g. bile salt hydrolase activity and thus poor fat digestion), a reduced inflammatory reaction because of the decreased pathogen load, amongst others (Butaye et al., 2003; Knarreborg et al., 2004). Also direct immune-modulatory effects by AGPs have been suggested. Whatever the mechanism of action is, it is evident that host-microbiota interactions are involved. The gut-microbiota interactions are very complex since the gut is an organ that contains multiple cell types that fulfill many functions, and is hosting a diverse microbiota that carries out many functions as well, including breakdown of dietary molecules and consequently production of absorbable end products, and maturation and development of the (mucosal) immune system. The term 'gut ecosystem' is used to describe that the gut and the gut microbiota are forming one organ, with specific functions that are derived from both the gut microbiota's genetic potential (the microbiome), and the functions of the host gut wall. Novel technologies (-omics technologies) have been used recently to get a better understanding of the host-microbiota interactions. More specifically, various studies using 16S rDNA sequencing led to the identification of microbial taxa that are associated with beneficial or harmful host responses, and metabolomics has been used to identify microbial metabolites that trigger these effects. The production of microbial metabolites can be steered using nutritional factors, creating an excellent opportunity to make animals more resilient against non-infectious and infectious challenges.

The host side: epithelial cells as major signal sensors

There are obvious anatomical and morphological differences between the gastro-intestinal tracts of poultry, pigs and cattle, with the latter being distinct because of the presence of forestomaches. While the rumen is a key fermentation chamber in adult cattle, calves fed on liquid diets (veal calves) do not develop forestomaches and can be considered as monogastrics, similar to chickens and pigs. Despite differences in anatomy and morphology, the cell types present in the gut wall are similar in these animal species. The luminal side of the intestinal wall is lined with absorptive epithelial cells, whose major task is water and nutrient uptake, and secretion of enzymes. They form a semi-permeable barrier between the outside world (the gut lumen) and the internal host tissues. The semi-permeable barrier is not only formed by the cell membranes of the epithelial cells, but also by tight junctions that connect neighboring epithelial cells (Piche, 2014). These connections are regulated at different levels (e.g. by cytokines). The permeability of the intestinal epithelial cell layer can be affected by epithelial cell death but also by luminal signals that increase the epithelial layer permeability by affecting the tight junctions or inducing cell death, and thus causing loss of integrity of an important barrier between the 'inside' and the 'outside' of the gut (Hooper, 2015). Loss of intestinal epithelial integrity can cause losses of host proteins ('leaky gut') into the lumen and can allow luminal molecules (including toxins) and micro-organisms to reach the gut submucosa under the epithelial layer. If these components have pro-inflammatory properties, this can yield massive infiltration of immune cells, which is energy-demanding for the host. Inflammation is mediated by binding of pathogen associated molecular patterns (e.g. LPS, peptidoglycan lipoproteins, flagellin) to receptors (e.g. Toll like receptors (TLRs)) that transmit signals in a cascade ultimately leading to inflammatory cell infiltration in the mucosa (Brown et al., 2011). Although this is a protective response, this inflammatory cascade should be brought back to normal conditions when the

trigger is eliminated. Also intracellular receptors (NOD-like receptors) can sense bacterial compounds and can even induce tolerance (eg. peptidoglycan-derived muramyldipeptides (MDPs)). Apart from absorptive epithelial cells, also other epithelial cell types are present in the lining of the gut wall. These include mucin-producing goblet cells and antimicrobial peptide producing Paneth cells (in the crypts, not present in all animal species), important in innate defenses (Muniz et al., 2012). Entero-endocrine cells can secrete peptide hormones at the basal side of the cells that can reach the bloodstream. These peptide hormones have a variety of functions, including effects on epithelial cell proliferation, inflammation, and consequently intestinal integrity, even at distant segments of the intestine. One of the key hormones is glucagon-like peptide 2 (GLP-2), a hormone that is important in maintaining epithelial integrity (Baldassano and Amato, 2014). Entero-endocrine cells are responsive to various luminal signals, including microbial signals. Below the epithelial lining, many other cell types are present that form the lamina propria of the intestinal mucosa. These are immune cells, fibroblasts, nerve cells and muscle cells, amongst others. Intestinal integrity, inflammation and gut function all are influenced by luminal signals, of which many are produced by the microbiota (Havenaar, 2011). The above mentioned cells sense microbial signals and transmit these signals to other cell types and to other parts in the body of the animal. The microbiota composition and the metabolites produced by the bacteria are thus crucial for health and productivity.

The microbial side: the microbiota as signal producers

The microbiota composition in the gut varies with age and with the gastrointestinal segment (Stanley et al., 2014; Song et al., 2017; Sun et al., 2019; Yang et al., 2019). In general the diversity of the microbiota increases with age. In industrial animal production, often the birth or hatching environment is as sterile as possible. This situation can be considered as unfavorable because the establishment of a protective microbiota is delayed, and the young animals are more prone to colonization by pathogens. The microbiota composition varies between the different segments of the gut. In general low numbers of bacteria are found in the proximal parts of the gut while the numbers increase towards the distal ileum, caecum or colon. The rumen of cattle is an exception, as this part is the main fermentation chamber in this animal species. The diversity generally increases significantly towards the distal gut (exception is rumen), and while in the small intestine a limited variability is found, with lactobacilli often dominant, the distal intestinal tract harbors a huge number of different bacterial groups. The distal intestinal tract of healthy subjects is mostly dominated by bacteria from the phyla *Bacteroidetes* and *Firmicutes* (together comprising more than 80% of the microbiota), the former containing many polysaccharide degrading bacterial species, while the latter contains a variety of bacterial families, including *Ruminococcaceae* and *Lachnospiraceae* families, that are considered important health-promoting populations, due to butyrate production. Also members of the phylum *Proteobacteria* are mostly present, although in lower numbers. These include *Enterobacteriaceae*, such as *Escherichia coli*, thus Gram-negative bacteria that contain opportunistic pathogens and often are associated with harmful inflammatory effects. The bacterial community has the genetic potential to carry out an enormous number of physiological functions. The number of microbial genes in the gut, the microbiome, exceeds the number of animal genes, and together they form a 'hologenome' (Rosenberg and Zilber-Rosenberg, 2011). The variety of bacterial functions includes degradation of complex substrates (polysaccharides, proteins, fat), fermentation of substrates to yield acidic compounds, immunomodulation, communication with other bacteria, and many more. The

metabolites produced by the bacterial community are of vital importance for maintaining gut health and controlling pathogen colonization.

Polysaccharide breakdown is performed by the microbiota in a cascade in which different bacterial members take care of specific catalytic steps in degrading the substrates (Flint et al., 2012). Complex substrates (such as polysaccharides, including arabinoxylans, pectins, and cellulose) are converted to oligosaccharides by specific bacterial populations (e.g. lactobacilli, some *Bacteroides* species, and others), and these oligosaccharides (e.g. arabinoxylanoligosaccharides (AXOS)) are further used by other bacterial groups to produce short-chain fatty acids (SCFAs, i.e. acetic, propionic and butyric acid), lactate and gases. Some polysaccharides can be broken down to glucose by single species. An example is cellulose breakdown by *Ruminococcus* spp. due to the production of extracellular enzymatically active protein complexes, called cellulosomes (La Reau and Suen, 2018). Depending on the bacterial network or the bacterial strains that carry out these conversions, other end products can be produced.

Short-chain fatty acids are the best known end products of polysaccharide fermentation. Apart from the typical acids, acetic, propionic and butyric acid, also succinate and lactate can be produced. The most important butyric acid producing bacteria belong to the *Ruminococcaceae* (Clostridial cluster IV) and *Lachnospiraceae* (Clostridial cluster XIVa) families (Pryde et al., 2002). These families contain strictly anaerobic bacteria that are highly abundant in the distal gut. Some of the *Lachnospiraceae* consume lactic acid to produce butyric acid (Duncan et al., 2004). Butyric acid has a variety of beneficial properties, including pathogen control, anti-inflammatory effects, increased mucin and antimicrobial peptide production, strengthening of the epithelial barrier, etc. (Guilloteau et al., 2010). Fermentation to butyrate in the distal gut can affect small intestinal function by stimulating GLP-2 secretion by entero-endocrine cells in the blood stream (Tappenden et al., 2003). This GLP-2 can have effects on various cell types in the small intestine, leading to anti-inflammatory effects, effects on the integrity of the epithelial barrier and increased cell proliferation (Rowland and Brubaker, 2011). Also proteins can be enzymatically broken down by the microbiota when they arrive in the distal compartment of the intestine (only possible when protein digestion by the host through pepsin, trypsin and peptidases did not completely degrade protein into amino acids for absorption. This can yield branched-chain fatty acids and amines, and the nature of these components depends on the amino acids being used by the bacteria. The effects of these specific metabolites (eg. polyamines spermidine, cadaverine, but also indoles) on host cells are not always clear, but some of these seem to have effects on transepithelial resistance as well. If an unfavorable shift in the microbiota composition occurs (= dysbiosis) certain crucial steps in certain pathways may be deficient, yielding shifts in bacterial metabolites in the gut. In general, there is a complex interaction between different bacterial populations for specific substrates, and the outcome of this competition can drive the microbiota to one that produces beneficial metabolites that promote gut health or toxic metabolites. When epithelial cells are killed or when the tight junctions between epithelial cells are damaged (e.g. by hydrogen sulphide, toxins, parasites), some opportunistic pathogens can take benefit by gaining access to the basolateral side of the epithelial cells and induce inflammation. Nutrient leakage and inflammation will cost energy for the animal, and will cause villus shortening or blunting, decreasing performance. When butyrate-producing bacteria are present in high amounts, the epithelial barrier integrity will in general be strong, the epithelial

proliferation and thus the villus length optimal and inflammatory reactions will be reduced, while the stimulation of regulatory T-lymphocytes will yield a state of tolerance to non-harmful bacteria. It is believed that the mucosa-associated microbiota is very important, as the bacteria and their metabolites are in intimate contact with host cells.

Interfering with bacterial signal production and host sensing, by nutritional interventions

A variety of feed additives are used nowadays as either antimicrobial growth promotor alternatives or gut health stabilizers. Some might inhibit certain bacterial groups but most are supposed to steer the microbiota composition to a more favorable one, and have important host effects, either direct or indirect, the latter through the microbiota. As discussed above, feed formulas or feed additives should improve intestinal epithelial integrity, stimulate tolerance responses towards non-harmful bacteria, avoid an excess inflammation, stimulate host antibacterial responses (mucin and antimicrobial peptide production) and bring the host in a steady state of mutualism with its microbiota. This means that these feed additives or formulae should favor beneficial microbes and inhibit the microbes that produce harmful metabolites, or reduce pathogen colonization. Below a short overview is given on dietary additives that affect gut health.

a) Feed composition and enzymes

Gut inflammation and villus shortening can be induced by feeding a diet containing high amounts of non-starch polysaccharides (NSP) without NSP-degrading enzymes (Teirlynck et al., 2009). AGPs are able to reverse the inflammatory changes and villus shortening induced by the high NSP containing diet, in association with a shift in the microbiota (Teirlynck et al., 2009). It appears that the use of AGPs in the past has masked the dysbiosis-inducing effects of many feed formulas used in monogastrics. Also the feed structure, protein source and the choice of ingredients can affect gut health. Enzymes such as xylanases convert large polysaccharides to shorter oligo-saccharides and thus perform one of the initial steps in the breakdown of these substrates, as is done in the gut by bacterial species in cross-feeding pathways. This also reduces viscosity and bacterial overgrowth in the small intestine. More info on the effect of feed constituents and gut health can be read in a review paper by Choct (2009), and in the paper of Kiarie in this proceedings book.

b) Probiotics

Probiotics are defined as live micro-organisms that, when consumed in adequate amounts, confer a health effect on the host. The most widely used bacterial probiotics are Bacilli, as they are stable in formulation (spores) and produce antibacterial compounds, apart from beneficial metabolites that are under discovery. As an example, recently it was shown in our lab that specific *Bacillus* species produce high concentrations of niacin *in vivo*. Niacin is sensed by the receptor Gpr109a, that is also activated by butyrate, and activates anti-inflammatory responses (Singh et al., 2014). Niacin has been shown to reduce epithelial apoptosis in the rumen of cattle induced by excess butyrate (Luo et al., 2019). Apart from Bacillus species, also other single strain probiotics are marketed, including lactobacilli. Multi-strain products are on the market as

well. Also competitive exclusion products, containing a freeze-dried mixture of gut content, are marketed. In the scientific literature, reports on the effect of probiotics on animal performance are published, and reports on the protection against pathogen colonization and disease are available. The question remains how many studies are not published because of inconsistent, no or negative effects observed. Data from our laboratory show that the efficacy of probiotics is highly depending on the model used and not all studies show clear reproducible beneficial results. Instead of empirically developing and marketing probiotics only because of their genus name, we should rethink the system and develop probiotics based on their mode of action. For example, based on the above described data, attempts could be made to evaluate strains that stimulate butyrate production by strains of Clostridial cluster IV and XIVa, or use these butyrate-producing strains as probiotics. These are, however, strict anaerobes and do not form spores consistently, while this is not a problem for *Bacillus* species, which are usually incorporated in feed as heat resistant spores (Shivaramaiah et al., 2011). For probiotic species that have consistent health effects, it would be good to identify mechanisms of action, and figure our which metabolites they produce that can exert direct or indirect effects on host health. This way new dietary additives can be developed.

c) Prebiotics

Prebiotics are defined as natural or processed functional foods which contain biologically active compounds that have documented benefits on health by altering the interactions between beneficial and pathogenic bacteria (Gibson and Roberfroid, 1995). Most prebiotics are oligosaccharides, such as fructooligosaccharides (FOS), galactooligosaccharides (GOS), arabinoxylan oligosaccharides (AXOS), xylan oligosaccharides (XOS) and raffinose family oligosaccharides (RFOs). Mannanoligosaccharides (MOS) are often not considered as prebiotics because they are not supposed to be fermented but have direct immunomodulatory effects. Prebiotics are complex molecules because of the chain length, the nature of the sugar bounds, and the nature of the side chains on the saccharides. All this can affect function. The scientific literature reports various studies in which prebiotics are having beneficial effects on broiler performance and pathogen control. As with probiotics, it is difficult to estimate the bias that is present using data derived from scientific papers, because only beneficial effects are mostly reported, and no or negative effects are seldom published. It is anyhow the case that the prebiotics need to be converted by the microbiota to metabolites. Because prebiotics are saccharides, the end products will be SCFAs, lactate and gases and thus the beneficial effect can theoretically be evaluated or predicted by measuring the ratio of beneficial versus harmful bacterial groups or metabolites. As such, prebiotics that increase colonization of butyrate-producing Clostridial cluster IV and XIVa bacteria are considered to be beneficial. Other parameters could include reductions in *Enterobacteriacea*. Also in the case of prebiotics we thus need to proceed in the future towards a science-driven development in which the mechanism of action plays a central role, instead of empirically developing prebiotics. For example, our group could show that XOS administration to a broiler diet increased the number of lactobacilli and Clostridial cluster XIVa strains in the distal gut, hereby stimulating cross-feeding of lactate to butyrate (De Maesschalck et al., 2015).

d) Synbiotics

Synbiotics are combinations of pre- and probiotics and thus offer the bacteria directly the substrate they can convert to beneficial metabolites (Roberfroid, 1998). In the above mentioned example, XOS and a Lactobacillus strain could for example be given to promote lactic acid production in the gut. This is a very simplified way of thinking because this depends on the fermentation of XOS by other bacteria at different sites in the gut, and the colonization site of the strain administered. There is thus a lot to learn on the nature of the substrates that are required to stimulate beneficial bacterial groups before these synbiotics can be developed with a high success rate.

e) Essential oils, phytobiotics

Essential oils and botanical products are also well-known feed additives in the animal production industry. Biologically active constituents of plants are terpenoids (mono-and sesquiterpenes, steroids, etc.), phenolics (tannins), glycosides and alkaloids (present as alcohols, aldehydes, ketones, esters, ethers, lactones, etc.). Many of these, but not all, have antibacterial activity (Penalver et al., 2005; Barbosa et al., 2009). Effects on immune function and host responses have not yet been investigated thoroughly, and also for this class of compounds it is difficult to explain the mechanism of action of the products that have been published in the scientific literature as beneficial for gut health. According to Adams (1999) the antimicrobial activity is rather weak for ginger and pepper, medium for cumin (p-cymene), coriander (lialol), oregano (carvacrol), rosemary (cineol), sage (cineol) and thyme (thymol) and strong for clove (eugenol), mustard (allylisothiocyanate), cinnamon (cinnamaldehyde) and garlic (allicin). Also here the dosage, purity, extraction method from the plant (in case of mixtures, thus phytobiotics) or synthetic production method will determine the success of the products. It is clear that the antibacterial essential oils will affect the gut microbiota composition, and there is a need to clarify which ones are promoting beneficial bacterial species, using *in vivo* studies. Resin acids have recently been studied and seem to alter matrix metalloproteinase activity in the gut mucosa, that could be highly relevant in restoration of intestinal damage (Aguirre et al., 2019). Indeed, matrix metalloproteinase upregulation has been shown in gut inflammation models and are likely involved in extracellular matrix breakdown.

f) Short chain fatty (and other) acids

Drinking water and feed additives containing SCFAs, medium chain fatty acids and even aromatic acids (e.g. benzoic acid) are widely used in the animal production industry. While drinking water acidification is mainly for sanitation purposes, feed additives are used mainly for optimizing animal performance and for pathogen control (Van Immerseel et al., 2006). It is difficult to compare the relative efficacy of commercial products because they differ in the nature of the acids used (often combinations are used), the concentration and even more important the delivery method (pure, on a carrier, encapsulated …). The latter determines the site of release in the gut and can affect the outcome. While SCFAs are more considered as signaling molecules for the microbiota and the host, the medium-chain and aromatic acids are more antibacterial.

Final considerations

A huge number of experimental and field trials have been carried out using a variety of feed additives, in production animals. The most commonly measured outcome parameter is performance, either or not under challenge conditions. Some studies have been undertaken to determine the effect on pathogen colonization. The approach so far was mostly empirical and the products are thus mainly developed without a clear understanding of the reasons of the expected beneficial effects. Many feed additives that are meant to replace AGPs have variable activities. The only way to develop a product with an enhanced activity as compared to the already existing products will be based on a thorough understanding of the intestinal ecosystem, and the way the gut wall responds to the microbiota and their metabolites. Identifying the microbiota components that are crucial for gut health is ongoing and is essential for proper development of additives that affect gut health. This needs to be done by identifying both the beneficial ones and the harmful ones. In fact current knowledge indicates that butyrate-producing bacteria need to be boosted or maintained while *Enterobacteriaceae* and specific pathogens such as *C. perfringens* need to be suppressed. These are easy to measure criteria and are well known to correlate with a good morphological structure of the gut. In fact, studies that are recently carried out and future studies using –omics technologies will be of value to identify potential performance-related beneficial gut microbiota components and metabolites (Torok et al., 2011). Although meta–omics tools (metagenomics, metabolomics, metaproteomics) can be very informative, there is a need to investigate the specific effect of the different bacteria and metabolites that are found to play a role in gut health. So bacterial culturing is a crucial tool to foster our understanding of the intestinal ecosystem and is essential to study the effect of a specific strain or species on gut health parameters (Walker et al., 2014). Only a small minority of the microbial species residing in the gut have been cultured so far, and thus isolation and characterization of new bacterial species from the gut will yield useful information. A recently established method to isolate, describe and genome sequence novel strains is called culturomics (Lagier et al., 2012). This method consists of plating gut or faecal samples on a variety (over 100) of media, followed by identification of the bacteria. Although this technique is able to discover new bacterial strains, a major breakthrough would be a method that permits to isolate strains that produce predefined metabolites, but this is not available yet. For the time being we have to rely on the isolation of potentially beneficial microbes and test their behavior, and more importantly study how we can promote their abundance in the animal gut. In the future many more health promoting and harmful bacterial groups and their respective metabolites will evidently be discovered and will steer our ways to optimize gut health and animal performance.

References

Adams, C., 1999. Nutricines: Food components in health and nutrition. Nottingham University Press, UK.

Aguirre M, Vuorenmaa J, Valkonen E, Kettunen H, Callens C, Haesebrouck F, Ducatelle R, Van Immerseel F, Goossens E. In-feed resin acids reduce matrix metalloproteinase activity in the ileal mucosa of healthy broilers without inducing major effects on the gut microbiota. Vet Res. 2019 Feb 22;50(1):15.

Baldassano S, Amato A. GLP-2: what do we know? What are we going to discover? Regul Pept. 2014 Nov;194-195:6-10.

Barbosa LN, Rall VL, Fernandes AA, Ushimaru PI, da Silva Probst I, Fernandes A Jr. Essential oils against foodborne pathogens and spoilage bacteria in minced meat. Foodborne Pathog Dis. 2009 Jul-Aug;6(6):725-8.

Brown J, Wang H, Hajishengallis GN, Martin M.TLR-signaling networks: an integration of adaptor molecules, kinases, and cross-talk. J Dent Res. 2011 Apr;90(4):417-27.

Butaye P, Devriese LA, Haesebrouck F. Antimicrobial growth promoters used in animal feed: effects of less well known antibiotics on gram-positive bacteria. Clin Microbiol Rev. 2003 Apr;16(2):175-88.

Choct M. Managing gut health through nutrition. Br Poult Sci. 2009 Jan;50(1):9-15

De Maesschalck C, Eeckhaut V, Maertens L, De Lange L, Marchal L, Nezer C, De Baere S, Croubels S, Daube G, Dewulf J, Haesebrouck F, Ducatelle R, Taminau B, Van Immerseel F. Effects of Xylo-Oligosaccharides on Broiler Chicken Performance and Microbiota. Appl Environ Microbiol. 2015 Sep 1;81(17):5880-8.

Flint HJ, Scott KP, Duncan SH, Louis P, Forano E. Microbial degradation of complex carbohydrates in the gut. Gut Microbes. 2012 Jul-Aug;3(4):289-306.

Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr. 1995 Jun;125(6):1401-12.

Guilloteau P, Martin L, Eeckhaut V, Ducatelle R, Zabielski R, Van Immerseel F. From the gut to the peripheral tissues: the multiple effects of butyrate. Nutr Res Rev. 2010 Dec;23(2):366-84.

Havenaar R. Intestinal health functions of colonic microbial metabolites: a review. Benef Microbes. 2011 Jun;2(2):103-14.

Hooper LV. Epithelial cell contributions to intestinal immunity. Adv Immunol. 2015;126:129-72.

Knarreborg A, Lauridsen C, Engberg RM, Jensen SK. Dietary antibiotic growth promoters enhance the bioavailability of alpha-tocopheryl acetate in broilers by altering lipid absorption. J Nutr. 2004 Jun;134(6):1487-92.

Lagier JC, Million M, Hugon P, Armougom F, Raoult D. Human gut microbiota: repertoire and variations. Front Cell Infect Microbiol. 2012 Nov 2;2:136. PMID:

La Reau AJ, Suen G. The Ruminococci: key symbionts of the gut ecosystem. J Microbiol. 2018 Mar;56(3):199-208.

Luo D, Peng Z, Yang L, Qu M, Xiong X, Xu L, Zhao X, Pan K, Ouyang K. Niacin Protects against Butyrate-Induced Apoptosis in Rumen Epithelial Cells. Oxid Med Cell Longev. 2019 Oct 13;2019:2179738.

Muniz LR, Knosp C, Yeretssian G. Intestinal antimicrobial peptides during homeostasis, infection, and disease. Front Immunol. 2012 Oct 9;3:310.

Peñalver P, Huerta B, Borge C, Astorga R, Romero R, Perea A. Antimicrobial activity of five essential oils against origin strains of the Enterobacteriaceae family. APMIS. 2005 Jan;113(1):1-6.

Pharmacol Res. 2013 Mar;69(1):52-60.

Piche T. Tight junctions and IBS--the link between epithelial permeability, low-grade inflammation, and symptom generation? Neurogastroenterol Motil. 2014 Mar;26(3):296-302.

Pryde SE, Duncan SH, Hold GL, Stewart CS, Flint HJ. The microbiology of butyrate formation in the human colon. FEMS Microbiol Lett. 2002 Dec 17;217(2):133-9.

Roberfroid MB. Prebiotics and synbiotics: concepts and nutritional properties. Br J Nutr. 1998 Oct;80(4):S197-202.

Rosenberg E, Zilber-Rosenberg I. Symbiosis and development: the hologenome concept. Birth Defects Res C Embryo Today. 2011 Mar;93(1):56-66.

Rowland KJ, Brubaker PL. The "cryptic" mechanism of action of glucagon-like peptide-2. Am J Physiol Gastrointest Liver Physiol. 2011 Jul;301(1):G1-8. Review.

Shivaramaiah S, Pumford NR, Morgan MJ, Wolfenden RE, Wolfenden AD, Torres-Rodríguez A, Hargis BM, Téllez G. Evaluation of Bacillus species as potential candidates for direct-fed microbials in commercial poultry. Poult Sci. 2011 Jul;90(7):1574-80.

Singh N, Gurav A, Sivaprakasam S, Brady E, Padia R, Shi H, Thangaraju M, Prasad PD, Manicassamy S, Munn DH, Lee JR, Offermanns S, Ganapathy V. Activation of Gpr109a, receptor for niacin and the commensal metabolite butyrate, suppresses colonic inflammation and carcinogenesis. Immunity. 2014 Jan 16;40(1):128-39.

Song Y, Malmuthuge N, Steele MA, Guan LL. Shift of hindgut microbiota and microbial short chain fatty acids profiles in dairy calves from birth to pre-weaning. FEMS Microbiol Ecol. 2018 Mar 1;94(3).

Stanley D, Hughes RJ, Moore RJ. Microbiota of the chicken gastrointestinal tract: influence on health, productivity and disease. Appl Microbiol Biotechnol. 2014 May;98(10):4301-10.

Sun J, Du L, Li X, Zhong H, Ding Y, Liu Z, Ge L. Identification of the core bacteria in rectums of diarrheic and non-diarrheic piglets. Sci Rep. 2019 Dec 10;9(1):18675.

Tappenden KA, Albin DM, Bartholome AL, Mangian HF. Glucagon-like peptide-2 and short-chain fatty acids: a new twist to an old story. J Nutr. 2003 Nov;133(11):3717-20.

Teirlynck E, Bjerrum L, Eeckhaut V, Huygebaert G, Pasmans F, Haesebrouck F, Dewulf J, Ducatelle R, Van Immerseel F. The cereal type in feed influences gut wall morphology and intestinal immune cell infiltration in broiler chickens. Br J Nutr. 2009 Nov;102(10):1453-61. PMID:

Torok VA, Hughes RJ, Mikkelsen LL, Perez-Maldonado R, Balding K, MacAlpine R, Percy NJ, Ophel-Keller K. Identification and characterization of potential performance-related gut microbiotas in broiler chickens across various feeding trials. Appl Environ Microbiol. 2011 Sep;77(17):5868-78.

Van Immerseel F, Russell JB, Flythe MD, Gantois I, Timbermont L, Pasmans F, Haesebrouck F, Ducatelle R. The use of organic acids to combat Salmonella in poultry: a mechanistic explanation of the efficacy. Avian Pathol. 2006 Jun;35(3):182-8.

Walker AW, Duncan SH, Louis P, Flint HJ. Phylogeny, culturing, and metagenomics of the human gut microbiota. Trends Microbiol. 2014 May;22(5):267-74.

Yang Q, Huang X, Wang P, Yan Z, Sun W, Zhao S, Gun S. Longitudinal development of the gut microbiota in healthy and diarrheic piglets induced by age-related dietary changes. Microbiology open. 2019 Dec;8(12):e923.

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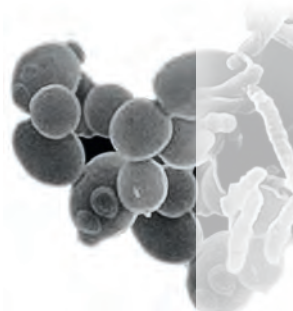
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Evaluating the effectiveness of *Lactobacillus Zeae* against enterotoxigenic *Escherichia coli* F4 infection with an *in vitro* intestinal porcine epithelial cell model

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Abstract

Lactobacillus Zeae (LB1), originally isolated from chicken, has been shown to have a high protective effect against infection of enterotoxigenic *Escherichia coli* (ETEC) in an *in vivo C. elegan* model. However, the underlying molecular mechanism is still unclear. In the present study, IPEC-J2 was used as an *in vitro* epithelial cell model and the protective effects of LB1 against ETEC F4 infection were investigated. The results showed that the pretreatment of IPEC-J2 cells with LB1 significantly alleviated the cytotoxicity and inhibited gene expression of inflammatory cytokines (interleukin IL-8 and IL-6) induced by the ETEC F4 challenge. The LB1 pretreatment maintained a significantly higher value of trans-epithelial electrical resistance (TEER) when challenged with ETEC F4 and a concomitant lower fluorescein isothiocyanate-dextran (FITC-dextran, MW 4,000) fluxes from apical side to the basolateral side compared with that challenged with ETEC F4 alone, which indicated that LB1 pretreatment protected barrier integrity. Results from ZO-1 and beta-actin staining showed that LB1 pretreatment could prevent tight junction and cytoskeleton damage caused by the ETEC F4 challenge, indicating that LB could keep tight junction structural integrity. However, LB1 inclusion did not show significant protection on tight junction protein ZO-1 and occludin expression at both gene and protein levels from the ETEC F4 challenge. These results suggested that probiotics LB1 could effectively protect epithelial cells against the ETEC F4 infection by inhibition of inflammation and maintaining barrier integrity. Our work allows gaining insight into the mechanisms that probiotics could exert to improve the host gut health.

The effects of partially replacing animal proteins with full fat black soldier fly larvae meal in nursery diets on growth performance and indices of immune system robustness of newly weaned pigs

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Abstract

Black soldier fly larvae meal (**BSFLM**) is enriched with both chitin and medium chain fatty acids (**MCFA**). Chitin acts as an immune stimulant and prebiotic to support a balanced and diverse population of gut microbes while MCFA exhibit anti-inflammatory and antimicrobial properties. This makes BSFLM a promising alternative to in-feed antibiotics in the diets of newly weaned pigs. One hundred and forty-four newly weaned pigs (6.73 ± 0.39 kg initial BW; 21 days of age) were placed in 24 pens (6 pigs per pen) and randomly assigned to one of four dietary treatments (study d 0; n=6), which were fed over 3 phases (phases 1, 2, and 3 were fed for 7, 14, and 21d, respectively). Two nursery diets were formulated with 25% (**LowFF**) and 50% (**HighFF**) of animal protein sources replaced by full fat BSFLM and two commercial nursery diets were used as controls (**-CON** no antibiotics; **+CON** with 220mg Aureomycin per kg of complete feed). Individual pig BW and per pen feed disappearance were recorded weekly. On d 37, two pigs per pen underwent a delayed hypersensitivity test. Skin swelling was measured at 0, 6, 24, and 48hr post-intradermal injection with antigens OVA and CAA as indices of humoral and cell-mediated immune responses, respectively. Pigs fed the -CON had lower ADFI and G:F in phase 2 versus those fed +CON or the BSFLM diets ($P<0.05$ and $P<0.05$). Overall ADG, ADFI, G:F, and final BW (27.64 ± 0.88 kg) were not affected by dietary treatments. Pigs fed the +CON, LowFF, and HighFF diets had a more robust immune response to OVA at 6hr than those fed the –CON diet ($P=0.078$, $P<0.05$, and $P<0.05$, respectively). Both inclusion levels of BSFLM in nursery diets supported growth performance, and indices of immune function not different from the +CON. Therefore, BSFLM may be a viable alternative to include in nursery diets as a replacement for in-feed antibiotics.

Key words: black soldier fly larvae meal, pig, antibiotics, immune system robustness

Expression of umami receptors (T1R1/T1R3) in the intestine during the embryonic and post-hatch development in broiler chickens

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Abstract

Umami receptors T1R1/T1R3 can recognize protein breakdown products including amino acids and peptides. Our previous results demonstrated that umami receptors T1R1/T1R3 are widely expressed in both the oral cavity and intestine of broiler chickens and the jejunum has a higher expression level of umami receptors within the intestine. However, the gene expression of umami receptors T1R1/T1R3 in the intestine during the embryonic and post-hatch development in chickens is still unknown. This study was to investigate the gene expression of T1R1/T1R3 in the intestine during the late embryonic and post-hatch development in broiler chickens. A total of 15 fertilized broiler eggs and 20 healthy broilers were used in this study. The jejunum was collected from 3 embryonic broiler chickens at the embryonic day (E) 17, 18, 19, 20 and 21. Four chickens were randomly sacrificed at 1 week, 2 weeks, 3 weeks, 4 weeks, and 5 weeks, and duodenum, jejunum, ileum, and colon were collected. We found the T1R1/T1R3 mRNA abundance was detected in the jejunum at all stages of the late embryonic development. The jejunal T1R1/T1R3 mRNA abundance was increased with the ages during the late embryonic development in chickens. The expression of T1R1/T1R3 in the duodenum, jejunum, ileum, and colon of chicks of different ages were varied. These results demonstrated that chicken umami receptors T1R1/T1R3 are expressed in the intestine of the late embryonic development and post-hatch development and their expression levels may be affected by the ages of embryonic development and post-hatch development. However, the roles of chicken umami receptors T1R1/T1R3 in the intestine are still not clear. Further studies are needed to investigate potential ligands of chicken umami receptors and cell signaling pathways activated by potential umami substances.

Keywords: chicken umami receptors T1R1/T1R3, gene expression, intestine, gut chemosensing, chickens

Modulation of bone mineral content in replacement gilts in response to a depletion and repletion protocol

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Abstract

To limit the use of mineral phosphates, there is an increased interest to feed phosphorus (P) levels allowing maximized growth, without fulfilling the higher requirement for maximised bone mineralisation to growing pigs. In some contexts of production, replacement gilts are raised with fattening pigs until 80-100 kg BW. Considering that bone mineralization of replacement gilts should be maximized to expect a high lifetime breeding performance, it was hypothesized that after a depletion period that render them more efficient to use Ca and P, they can recover bone mineralisation when fed a repletion diet. From 58-95 kg BW, twenty-four Swiss Large White gilts were fed either a finisher control diet (C; 2.1 g digestible P/kg) or a finisher low P diet (L; 1.2 g digestible P/kg). From 95-140 kg BW, half of the gilts from each finisher diet were randomly assigned to either a C diet or a high P diet (H; 3.5 g digestible P/kg), resulting in four treatments: LC, CC, LH and CH. The Ca:dig. P ratio was 3.3, 3.1 and 2,9 for C, L and H diets, respectively. Whole body bone mineral content (BMC) as well as body composition of pigs were measured on each gilt at two-week intervals by dual energy X-ray absorptiometry. Data were analyzed as a randomized complete block design with PROC MIXED of SAS. Diet did not affect growth performance during the entire experiment. At 95 kg, gilts fed L showed a reduced BMC (8.7%; $P<0.001$) compared to the gilts fed C. At 140 kg, BMC of gilts fed LC were similar to CC, but lower than LH (2989 and 3133 vs 3314 g; $P<0.001$) while BMC of gilts fed LH and CH were not different (3314 vs 3356 g). These results show the important potential to limit dietary digestible P concentration during the fattening period without revealing detrimental effects on growth performance and they confirm the ability of replacement gilts to recover their bone mineralisation by 140 kg BW by increasing BMC efficiency. Finally, a high dig. P content between 95-140 kg BW allowed to further increase bone mineralisation, but required the use of dietary phosphates.

Key words: replacement gilts, repletion, depletion, phosphorus, calcium, bone mineralization

Comparative Evaluation of Organ Weights and Breast Meat Yield of Commercially Reared Broiler Chickens on Conventional and Alternative Feeding Programs

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Abstract

There are concerns that antibiotics used in human medicine are no longer as effective in the treatment of disease due to the perception of overuse in animal agriculture. Consequently, the agricultural industry must identify alternative feeding strategies that do not rely on antibiotics. Overall health and performance in response to elimination of antibiotics has been reported but the physiological response to elimination as a component of commercial feeding programs remains unclear. The objective of this study was to evaluate the impact of different antibiotic regimes on organ weight and breast meat yield in commercially reared broilers. Parameters were compared between barns allocated to conventional, antibiotic-reduced or antibiotic-free (RWA) programs across Ontario. At 28 days of age, 288 clinically normal broilers were euthanized over 4 consecutive quota periods and body weight, organ weights and breast yield evaluated. Data was analyzed to ensure residuals were normally distributed and an analysis of variance was conducted on the effects of treatments over time (Proc Glimmix, SAS 9.4). Feeding program did not ($P>0.05$) impact body or organ weights (gizzard, proventriculus, spleen, small intestine, and bursa). Breast yield ($P=0.006$) was significantly lower (-13%) for RWA birds compared to the other programs. Crop ($P=0.03$) and liver weight ($P=0.03$) were significantly larger in birds on the RWA program. In conclusions, reduction of breast yield in RWA birds may be due to reduced amino acid availability, moreover, RWA birds may be exposed to higher levels of antinutritional factors and pathological conditions leading to changes in organ structure and function. Additional investigations are required to quantify the differences throughout a broiler's life cycle to determine the impact on overall growth performance and economics.

Key words: broiler, antibiotics, organ weights, breast yield

Molecular Distribution and Localization of Calcium-sensing Receptor and Vitamin D Receptor in Laying Hens

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Abstract

Calcium-sensing receptor (CaSR) and vitamin D receptor (VDR) play important roles in regulating calcium mobilization, calcium absorption and maintaining calcium homeostasis, which could be potential therapeutic targets for osteoporosis in laying hens. The present study was to investigate the molecular distribution of CaSR and VDR, and the localization of CaSR in the kidney, proventriculus, duodenum, jejunum, ileum, colon, cecum, shell gland and tibia of laying hens at three different laying stages (19, 40 and 55 weeks). The RT-qPCR results showed that at the laying peak (40-week age), the relative mRNA abundance of CaSR in the kidney, ileum, proventriculus, duodenum, and colon was significantly higher than other tissues ($P <$ 0.05). The variation trend of 55-week age was consistent with that of 40-week age. In the tibia, the relative mRNA abundance of CaSR at 55 weeks of age was significantly higher than that at 40 weeks of age ($P < 0.05$). However, western-blotting results showed that no significant difference of relative CaSR expression was found in the different tissues at the laying peak, or in each tissue at three different laying stages ($P > 0.05$). The relative mRNA abundance of VDR was significantly higher in the small intestine (duodenum, jejunum, and ileum) when compared with other tissues at three different laying stages. Western blotting results showed that the expression of VDR in the duodenum was significantly higher than that in the proventriculus, colon, and cecum, respectively ($P < 0.05$). Different laying stages did not affect the VDR expression in each tissue ($P > 0.05$). From IHC results, we detected that the positive brown staining can be found widely in each tissue, and different laying stages did not affect the localization of CaSR except for the tibia tissue. In conclusion, CaSR and VDR are widely expressed in the gut, bone and shell gland of laying hens, but their expressions are rarely affected by the different laying stages in each tissue.

Energy contents of heat-treated and intact dry extruded-expelled soybean meal fed to growing pigs

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Abstract

Thermal processing is commonly performed to deactivate antinutritional factors in soybeans used as swine feed. One such process combines extrusion with expelling, resulting in dry extruded-expelled soybean meal (DESBM). For DESBM to be practically used as feed in the swine industry, its energy content must be accurately determined. Furthermore, information is limited concerning the effect heat damage during processing has on the energy content of soybean meal. Therefore, an experiment was conducted to determine the digestible energy (DE) and metabolizable energy (ME) contents of both heat-treated and intact DESBM. Eighteen growing barrows (18.03 ± 0.61 kg initial body weight) were individually housed in metabolism crates and randomly allotted to one of three dietary treatments to give six replicates per treatment. The three experimental diets were: a corn-soybean meal-based basal diet, two test diets with simple substitution of basal diet with intact DESBM or heat-treated DESBM in a 70:30 ratio. Intact DESBM was autoclaved at 120°C for 60 mins to make heat-treated DESBM (heat-DESBM). Pigs were fed the experimental diets for 16 d, including 10 d for adaptation and 6 d for total collection of feces and urine. Pigs were fed their assigned diets at 550 kcal ME/kg $BW^{0.60}$ per day (based on body weight on days 1 and 10), which was close to ad libitum intake. The energy contents of the tested DESBM were calculated by the difference method. All data were analyzed using the Mixed procedure of SAS with the individual pig as the experimental unit. Pigs fed the intact DESBM diet showed greater ($P < 0.01$) apparent total tract digestibility of dry matter (DM), gross energy (GE), and GE retention (%) than those fed heat-DESBM. This resulted in greater DE ($P < 0.01$) and ME ($P = 0.05$) in the DESBM diet than in the heat-DESBM diet. Therefore, intact DESBM had greater ($P < 0.05$) DE (4,591 kcal/kg DM vs. 4,222 kcal/kg DM) and ME (4,099 kcal/kg DM vs. 3,692 kcal/kg DM) contents than heat-DESBM. Pigs' lower energy use with heat-DESBM may be attributable to protein denaturation and insoluble protein caused by the Maillard reaction during heat processing. Therefore, it is recommended that heat damage be carefully considered when soybeans are processed into meal for swine feed.

Key words: dry extruded-expelled soybean meal, energy content, heat treatment, pigs

The metabolizable energy and standardized ileal digestibility of amino acids in dry extruded-expelled soybean meal in comparison with solvent-extracted soybean meal fed to broiler chickens

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Abstract

The thermal treatment during extrusion can inactivate antinutritional factors in soybeans. Expelling extraction leaves behind soybean meal with higher contents of oil than solvent extraction does. The combination of these processing techniques results in dry extruded-expelled soybean meal (DESBM). It is expected that the feeding value of DESBM is high for broiler chickens, making it important to determine the nutritive values of DESBM to be used in broiler diets in comparison with solvent-extracted soybean meal (solvent SBM). An experiment was conducted to determine the standardized ileal digestibility (SID) of amino acids (AA) and the apparent metabolizable energy (AMEn) contents of DESBM and solvent SBM. Two hundred and forty broiler chicks were divided into 40 groups of 6 birds balanced for body weight and fed 5 experimental diets in a completely randomized design (8 groups/diet) from age 14 to 21 d. Two basal diets were cornstarch-DESBM-based and cornstarch-solvent-SBM-based diet. A portion of cornstarch and SBM in each basal diet were replaced by 30% of DESBM for cornstarch-solvent-SBM basal diet and 30% of solvent-SBM for cornstarch-DESBM basal diet. A cornstarch-casein-based diet was also included to estimate basal endogenous AA losses for determining the SID of the AA. From d 19 to 21, excreta samples were collected for AMEn determination. On d 21, the birds were euthanized, and the contents of the ileums were obtained for determination of AA digestibility. The AMEn contents of SBM were determined by the substitution method, whereas the SID of the AA in SBM were directly determined from each basal diet. All data were analyzed using the mixed procedure of SAS. The apparent total tract retention of energy in the DESBM fed to broilers was lower ($P = 0.03$) than that in the solvent SBM. However, no difference in AMEn contents was observed between the DESBM (2,390 kcal/kg) and solvent SBM (2,314 kcal/kg). The SID of the AA in the DESBM and solvent SBM ranged between 79.6%–95.6% and 87.45%–94.2%, respectively. The SID of Ile, Leu, Met Ala, Asp, Gly, Ser, and Tyr in the solvent-SBM were greater ($P < 0.10$) than those in the DESBM. The higher dietary fiber content in DESBM than that in solvent SBM may lead to lower SID of AA and energy digestibility in broilers.

Key words: broiler chickens, dry extruded-expelled soybean meal, energy content, standardized ileal digestibility

Standardized ileal digestible lysine requirements for 7- to 11-kg weanling pigs fed a corn-soybean meal-based diet

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Abstract

Amino acid requirements are known to vary with pig genotype and using the cost estimates has economic as well as environmental implications. The objective of this research was to determine the standardized ileal digestible (**SID**) lysine (**Lys**) requirements for 7- to 11-kg weanling pigs (TN Tempo × TN70) fed a corn-soybean meal-based diet. A total of 144 piglets (6.51 ± 0.56 kg BW) were assigned to 1 of 6 diets using a randomized complete block design based on body weight to give 8 replicate pens with 3 pigs per pen. The six diets contained 1.00, 1.16, 1.32, 1.48, 1.64, and 1.80% SID Lys, respectively. Piglets had free access to diets and water for 14 days. Average daily gain (**ADG**) and average daily feed intake were not affected by dietary SID Lys content during the first 7 days, however, the addition of dietary SID Lys quadratically increased ($P < 0.05$) gain:feed (**G:F**) of piglets. A quadratic increase ($P < 0.05$) was found in ADG when SID Lys content increased in the diets from day 7 to 14. During the overall experimental period, increasing dietary Lys content quadratically increased ($P < 0.05$) ADG and G:F, whereas plasma urea nitrogen was quadratically decreased ($P < 0.05$) as SID Lys content increased. The SID Lys requirements for optimal growth performance of 7- to 11-kg weanling pigs fed corn-soybean meal-based diets ranged from 1.28 to 1.44% for ADG and 1.38 to 1.55% for G:F, respectively, thus giving an overall average value of 1.42%. These results contribute to understand more accurate Lys requirements in weaning pigs, which is required for optimal formulation of the swine diets. The determined Lys requirement value can be used to formulate diets that closely meet amino acid requirement for high lean pig genotypes. This has the added advantage of minimizing nitrogen excretion into the environment and saving the feed cost.

Key words: broken-line analysis, growth performance, lysine, piglets

Effects of Peptidoglycan on inflammation, nutrient transporter gene expression and barrier function in porcine intestinal epithelial cells (IPEC-J2 cells)

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Abstract

Peptidoglycan (PGN) is a polymer in bacterial cell walls and may constrain the gut functionality and lowering intestinal efficiencies in livestock. Previous studies demonstrated that dietary muramidase, an enzyme to cleave the β-1, 4 glycosidic linkages between N-acetylmuramic acid and N-acetyl glucosamine in the carbohydrate backbone of peptidoglycan, contributes to improving feed conversation ratio and gastrointestinal function in broiler chickens and swine. However, in the small intestine, how PGN regulates inflammatory response and influences nutrient absorption and barrier function are poorly elucidated in livestock. In this study, IPEC-J2, a jejunal epithelial cell line was used as an intestine epithelial cell model, and the effects of PGN on inflammation, nutrient transporter gene expression and barrier function were investigated by measuring gene and protein expression of cytokine IL-8 and TNFα, gene expression of nutrient transporters, gene expression of tight junction proteins and transepithelial electrical resistance (TEER). The results showed that PGN stimulation induced a significant amount of IL-8 and TNFα expression on both mRNA and protein levels ($P < 0.05$ compared with control) in a dose-dependent manner. PGN challenge also significantly reduced the mRNA abundance of sodium-dependent glucose transporter 1 (SGLT1) and glutamate transporter (EAAC1) while did not alter mRNA abundance of neutral amino acid transporter (B^0AT1), neutral amino acid exchanger (ASCT2) and peptide transporter 1 (PepT1). However, PGN treatment did not alter the mRNA abundance of Zonula occludes 1 (ZO-1) and claudin-3 and structural integrity of tight junction and cytoskeleton respectively. Furthermore, it also did not affect TEER value. These results suggest that infection of gram-positive bacteria such as Staphylococcus aureus may induce inflammation in the intestine epithelial cells and compromise some nutrient absorption.

The Effect of Incremental Levels of Omega-3 Fatty Acids from either Flaxseed Oil or Preformed Docosahexaenoic (DHA) on Health Indices of Laying Hens

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Abstract

Dietary provision of omega-3 polyunsaturated fatty acids (PUFA), including alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), has received considerable attention, including in poultry nutrition. The latter has primarily focused on producing omega-3 enriched eggs and meat for human consumption. The current study, however, was designed to determine potential health benefits to laying hens receiving different sources of dietary omega-3 fatty acids, as assessed during an inflammatory challenge induced by lipopolysaccharide (LPS) administration. A total of 80 Lohmann LSL-Classic (white egg layer) were randomly assigned to 1 of 8 treatment diets (10 hens/treatment), with diets formulated to meet NRC requirements for laying hens. Treatments provided 0.2%, 0.4%, 0.6%, or 0.8% content of total dietary omega-3 fatty acids, provided as either flaxseed oil (ALA) or algal DHA for 56 days. Specific measures included weekly body weight, feed intake, egg production, egg weights and egg quality measures. On day 56, birds from each treatment were injected with either saline (Sham; n=5) or LPS (8 mg/kg i.v.; n=5), and blood and tissue samples (post-CO_2 asphyxiation) were collected for subsequent analysis of fatty acids and oxylipins. In general, dietary omega-3 supplementation patterns yielded predictable responses in plasma, liver and yolk fatty acid concentrations in response to increasing dietary ALA or DHA. LPS challenge led to significant reductions in mean liver EPA (mg/g; Sham = 0.088 mg/g +/-0.004; LPS = 0.077+/-0.004 mg/g; $P<0.05$). There was a significant Diet×Stress interaction for plasma EPA and DHA (mg/ml). Analysis of plasma oxylipins indicated significant main effects due to LPS and omega-3 supplementation for certain oxylipins (ng/ml), including a Stress effect on the EPA-derived 9-hydroxy-eicosapentaenoic acid (HEPE) (Sham = 0.013+/-0.008 ng/ml; LPS = 0.039+/-0.008 ng/ml; $P<0.05$). In summary, LPS challenge modulated certain oxylipins, including those derived from EPA. Further studies will clarify the role of dietary omega-3 fatty acids in modulating responses to an inflammatory challenge in laying hens and the biological significance of changes in oxylipin profiles. (NSERC/Manitoba Egg Farmers/Egg Farmers of Canada)

Effects of Epidermal Growth Factor (EGF) on intestinal organ development and growth performance in broiler chickens

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Abstract

Poultry producers are faced with challenge of producing poultry products without using preventive antibiotics. Epidermal Growth Factor (EGF) is a protein that regulates cell growth, differentiation, maturation and healing. Exogenous EGF promotes intestinal development in mice, rabbits and pigs but studies in poultry are scarce. This study's objective was to evaluate effects of EGF on organ development and growth performance in broiler chickens. A total of 912, d old male Ross 708 chicks were placed in floor pens (19 birds/pen) based on body weight (BW). Pens were assigned to 1 of 6 treatments: **A**- positive control with antibiotic and coccidiostat, **B**- negative control; no additive, **C**- Culture media used for EGF fermentation but free of EGF, **D**- 160µg/kg BW/d EGF, **E**- 320µg/kg BW/d EGF and **F**- 640µg/kg BW/d EGF. Treatments were incorporated in a common starter diet and fed *ad lib*. On d10, BW and feed intake (FI) were taken for BW gain (BWG) and feed conversion ratio (FCR) and 2 birds/pen necropsied for organ weight measurements. A common grower/ finisher diet was fed from d 11 to 35. On d 35, 2 birds/pen were necropsied for breast yield. Data was analysed as one-way ANOVA in R (3.5.2). In the starter phase, BWG of EGF fed birds was similar ($P>0.05$) to A and B birds. However, diet C birds had higher BWG ($P=0.005$) than B. EGF320µg birds had a lower ($P<0.0001$) feed intake than C, but similar to other treatments ($P>0.05$). Treatments had no ($P>0.05$) effect on overall (d 0-35) growth performance. EGF640µg birds had higher ($P=0.015$) small intestine weight than C whilst other treatments were intermediate. Gizzard, spleen, liver and breast weights were not affected ($P>0.05$) by treatments. Treatment C birds had larger ($P=0.01$) bursas relative to EGF320 µg birds. In conclusion, EGF did not affect overall growth performance and intestinal organ development. EGF effects are reportedly more apparent on damaged epithelial cells hence the need to evaluate effects in a disease- challenge model.

Key Words: EGF, antibiotics, broilers

Effects of red-osier dogwood extracts as an alternative to in-feed antibiotics on performance, intestinal microbiota, digestive and absorptive function and meat quality in broiler chickens

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Abstract

This study evaluated the efficacy of red-osier dogwood extracts (**RDE**) as an alternative to in-feed antibiotics on performance, intestinal digestive and absorptive function, intestinal microbiota and meat quality in broiler chickens. A total of 320 day-old Cobb 500 chicks (48.3 ± 3.3 g) were assigned to 4 dietary treatments (8 replicates per treatments) as follows:1) negative control (**NC**) corn-soybean basal diet, 2) positive control (**PC**) basal diet and 30 ppm avilamycin, 3) basal diet supplemented with 1000 ppm RDE (**RDE1**) 4) basal diet with 3000 ppm RDE (**RDE2**). There were no significant differences in average daily gain, average daily feed intake, feed conversion ratio and organ weights among treatments ($P > 0.05$). Intestinal morphology showed reduced jejunal crypt depth and increased villus: crypt ratio in groups treated with RDE ($P < 0.05$). Cationic amino acid transporter mRNA expression was lower ($P < 0.05$) in the RDE and PC treatments while peptide transporter and neutral amino acid transporter were highly expressed ($P < 0.05$) in broilers in the RDE2 treatment compared to the NC. Ileal crude fat digestibility was higher in the RDE and PC compared to the NC while the ileal digestibility of amino acids was higher in the RDE1, RDE2 and PC groups ($P < 0.05$). The relative abundance of the ileal bacteria of orders (Turicibacterales, Bacillales and Clostridiales and *Staphylococcus* species) differed among treatments. Species richness between bacterial communities in the ileum and cecum was affected by treatments ($P < 0.05$). Tight junction proteins zonula occludens-1, and E-cadherins were expressed lower in RDE1, RDE2 and PC ($P < 0.05$) while claudin 1 remained unchanged ($P > 0.05$). Meat quality showed no difference in pH, drip loss, myofibrillar fragmentation index, shear force, lipid peroxidation and cook traits ($P > 0.05$). However, RDE2 treatment showed reduced redness compared to the NC ($P < 0.05$). In conclusion, RDE improved the intestinal environment and functions by improving digestion, absorption, barrier functions and microbiota diversity.

Keywords: red-osier dogwood, broiler chickens, growth performance, digestibility, meat quality

The effect of multi-carbohydrase preparation on growth performance and nutrient utilization on broiler chickens fed diets with high inclusion of canola meal

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Abstract

Canola meal (CM) is a valuable source of protein for poultry, however it contains significant amount of non-starch polysaccharides (NSP). Supplementation of poultry diets containing CM with enzymes is an effective means of improving the quality of CM. The enzymatic degradation of NSP of diets containing CM will contribute to the production of NSP hydrolysis products, which may have a prebiotic effect in improving animal health and development. A series of the *in vitro* incubation studies were carried out to determine if various carbohydrase preparations can target NSP of CM. The most effective enzyme combination, expressing mainly the pectinase and xylanase activities, was studied further in a 3-wk (1-21 d of age) growth performance and NSP digestibility trial with broiler chickens. Broilers were assigned to 3 dietary treatments, each consisting of 8 pens of 5 birds each, and were fed a control corn/CM-based starter and grower diets, and diets supplemented with enzyme cocktail at two levels – 0.03% and 0.15%. In comparison to the control diets, birds fed the diet supplemented with the high concentration of carbohydrases showed significantly ($P<0.05$) higher body weight gain during the grower phase and the entire study. Carbohydrase enzyme combination had a significant effect on NSP digestibility ($P<0.05$), which increased from 0.12% for the control treatment to 19.4%, and 18.6% for the two enzyme-supplemented diets, respectively.
Our research suggests that administation of carbohydrase enzyme in diets with high inclusion of canola meal can improve growth performance and NSP digestibility in poultry.

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

"Does *In ovo* delivery of probiotic affect hatch and growth performance, and intestinal functionality in broiler chickens?"

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Abstract

As the poultry industry recedes from the use of antibiotic growth promoters, the need to evaluate the efficacy of possible alternatives and the delivery method that maximizes their effectiveness arises. This study aimed at expounding knowledge on the effect of the delivery method of a probiotic product (*Bacillus subtilis* fermentation extract) on performance and gut parameters in broiler chickens.

A total of 500 fertile eggs sourced from Cobb 500 broiler breeders were randomly allotted to 3 groups: *in ovo* probiotic, *in ovo* saline and non-injection and incubated for 21 days. On day 18.5 of incubation, 200 µl of either probiotic (10 million CFU) or saline was injected into the amnion. At hatch, chicks were re-allotted to 6 new treatment groups: *in ovo* probiotic, *in ovo* saline, in-feed antibiotics, in-water probiotics, in-feed probiotics and negative control (**NC**; Corn-wheat-soybean diet) in 6 replicate cages (n=7) and raised for 28 days.

Results showed treatments did not affect all hatch performance parameters and the incidence of Necrotic enteritis on d 28 ($P > 0.05$). Irrespective of delivery method, probiotics increased body weight, body weight gain and feed conversion ratio, relative to the NC treatment at d 0-21 ($P > 0.05$). At d 22-28, in-feed antibiotics treatment recorded higher mortality relative to other treatments ($P=0.05$). Ileum villus width of the *in ovo* probiotic treatment was 18% higher than the *in ovo* saline group ($P=0.05$), but not statistically higher than other groups. Jejunum villus height was 23% higher ($P=0.00$) in the *in ovo* probiotic group compared to the NC group. There was no effect of treatment on total cecal short-chain fatty acid concentration ($P > 0.05$).

Conclusively, the probiotic improved growth performance of broiler chickens, regardless of delivery method. *In ovo* delivery of the product enhanced intestinal morphology, without compromising hatch and growth performance.

Key words: *in ovo*, probiotics, performance, broiler chicken

Is there a synergic effect when corn distillers dried grains with solubles (DDGS) or wheat middlings (WM) are steeped with combination of carbohydrase and protease?

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Abstract

An *in vitro* study was conducted to determine effects of steeping two co-products, DDGS or WM, with carbohydrase or protease alone, and in combination. The four treatments were; 1) co-product steeped with water excluding enzymes (**CON**), 2) CON with carbohydrase (**FDE**) 3) CON with protease (**PRO**) 4) CON with carbohydrase and protease (**FDEPRO**). Co-product (50 g), 500 mL water and respective treatment was steeped for 72h at 37°C in incubator shaker. At 0, 12, 24, 48, and 72h; pH was measured, and samples of supernatant submitted for mono-sugars and organic acid and of solids for fiber disappearance. Mono-sugar increased at 12-24h followed by a decrease at 48-72h in both co-products. Steeping DDGS with FDE and FDEPRO showed greater concentration of arabinose and glucose for than PRO at 12 h whereas xylose concentration was greater for FDEPRO than other treatments. At 12h, WM steeped with PRO had lower concentration of arabinose and xylose compared with FDE and FDEPRO, however, only FDE had a greater glucose concentration than PRO. In both co-products, pH decreased while lactic and acetic acid increased overtime. At 72h, both co-products showed lower pH and greater lactic acid concentration in FDE and FDEPRO than PRO. Greater disappearance of NDF and ADF was seen at 48-72h of steeping. At 72h, fiber disappearance was greater for FDE and FDEPRO than CON and PRO for DDGS but not for WM. In conclusions, enzyme application showed differences in the two co-products, however, combination of carbohydrase and protease seemed not necessary to improve mono-sugar release and fiber breakdown in these co-products.

Key words: carbohydrase, corn DDGS, protease, steeping, wheat middlings

Feeding behaviour patterns do not explain the observed variation on lipid and protein gain in growing pigs fed ad-libitum

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Abstract

Large variability on the proportion of lipids and protein of the body composition is observed in pigs fed and raised in similar conditions. Part of this variation might be explained by changes on feeding behaviour due to its effect on metabolic hormones associated with energy regulation and protein metabolism. Therefore, this study focused on the creation of an index to describe feeding behaviour patterns and to study its relationship with body composition in growing pigs. Feeding behaviour and body composition information of 165 pigs during the last 28 d of the growing phase from three studies was used. For each pig, the sum of the areas between the observed relative cumulative feed intake (RCFI) and the calculated line obtained with a regression model of the evolution of RCFI over time was used to calculate a new index that illustrates the irregularity of feed intake (IFII). Correlations of IIFI with number of daily meals (NDM; r = -0.44; P < 0.001), duration of meal (DUM; r = 0.42; P < 0.01) and, feed intake per meal (FIM; r = 0.40; P < 0.01) indicate that pigs with high IFII have fewer meals of longer duration and higher feed intake compared with pigs having small IFII. It demonstrates that IFII integrates information of several components of the feeding behaviour of the pig. However, correlations between IFII and the % of protein and lipid of the body gain were weak and only significant in one study and accounted only for 12% of the total variation of body composition. In conclusion, other factors than feeding behaviour modulate body composition of growing pigs fed ad libitum. Nevertheless, the IFII obtained in this study combine different characteristics of the feeding behaviour, which allows studying in a more objective manner the relationship of the dynamic of the feed intake with other aspects of the animal performance, growth or health.

Key words: feeding behaviour, body composition, precision farming

Extrusion increased protein digestibility and energy value of lentil-based diets containing soybean meal or fish meal in growing pigs

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Abstract

Lentil grain is an energy and protein source for pigs. However, anti-nutritional factors and fibre in lentil may limit its inclusion in swine diets. Extrusion may increase its nutritional value, but whether plant or animal protein sources interact with lentil during extrusion is unknown. To explore, two diets were prepared: 1) soybean meal (SBM) diet, containing 50% lentil, 31% wheat and 12.8% SBM; 2) fish meal diet, containing 40% lentil, 45% wheat and 10% fish meal. Half of each diet was extruded using a single-screw extruder. The 2 extruded and 2 non-extruded diets were fed to 8 ileal-cannulated barrows (32.3 kg) for four 9-d periods in a double Latin square. Data were analyzed as a 2×2 factorial arrangement. Extrusion increased ($P < 0.05$) diet apparent ileal digestibility (AID) of crude protein (CP) and amino acids (AA) by 3.2 and 4.7%, and apparent total tract digestibility (ATTD) of gross energy (GE) by 2% and hence digestible energy (DE) values for SBM diet and fish meal diet, respectively. Interactions between protein sources and extrusion of diets were not observed. The fish meal diets had greater ($P < 0.05$) AID of dry matter (DM), GE, CP and all AA except methionine, and ATTD of CP, lower ($P < 0.05$) apparent hindgut fermentation (AHF) of DM and GE, but did not differ in ATTD of GE and AHF of CP compared with the SBM diets. Extruded SBM-lentil diet had comparable AID of CP and AA, and greater energy value compared with non-extruded fish-meal-lentil diet. In conclusion, extrusion increased the nutritional value of SBM-lentil and fish-meal-lentil diets. The protein digestibility of the extruded SBM-lentil diet was not different to that of the non-extruded fish meal diet. Thus, extrusion may help to reduce the inclusion of costly animal protein sources in swine diets. The finding is also relevant for the pet food industry that uses extrusion to produce kibbles.

Key words: extrusion, fish meal, lentil, pig, soybean meal

The potential activation of calcium sensing receptor by deoxynivalenol contamination in piglets and the effects of vitamin supplements (D, E and C)

L'activation potentielle du récepteur sensible au calcium par une contamination au désoxynivalénol chez des porcelets et l'effet d'une supplémentation en vitamine D, E et C

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Abstract

Diversification of feed ingredients by adding by-products or low-graded grains is a way to reduce feed cost in pig production. However, this strategy can introduce mycotoxins, especially deoxynivalenol (DON). DON has been showed to induce anorexia, immunomodulation, oxidative stress, and more recently, alterations of calcium (Ca) and phosphorus (P) metabolism. It was thus hypothesised that vitamin D_3, 25-OH-D_3, or vitamin C and E addition can counteract the effects of DON in piglets. A total of 96 piglets were divided into 48 pens of the 9 treatments in 3 x 3 factorial design: control treatments (CON), DON or DON+VitE-C, these three diets were unsupplemented or supplemented with VitD$_3$ or 25-OH-D$_3$. After 21 days, piglets were weighted and one per pen received LPS injection as an acute inflammatory reaction 3h prior to euthanasia. Bone mineral content of each piglet was measured with dual-intensity X-ray absorptiometry. Blood samples were taken to assess concentrations for VitD, E and C, P, Ca, antioxidant enzymes. Results showed that, DON or DON+VitE-C had lower average daily feed intake and average daily gain ($P<0.001$). DON also had higher BMC per kg live body weight (10.27%, $P<0.001$) than CON, while DON+VitE-C had an intermediate bone mineral content. DON and DON+VitE-C also had lower plasma concentrations of 25-OH-D_3, 1,25-(OH)$_2$-D_3 and phosphate ($P<0.001$). The superoxide dismutase activity in intestinal mucosa after LPS injection was reduced in DON and DON-VitE-C piglets, but in unstimulated LPS pigs, the activity was increased only in DON (LPS x DON, $P <0.002$). However, in intestinal mucosa, DON+VitE-C had lower catalase activity and MDA concentration than CON with intermediary values for DON ($P<0.05$). The DON induced intestinal and systemic modification of oxidative status and alteration of vitamin D-calcium-phosphorus metabolism. More studies are needed to assess the link between these phospho-calcium metabolism changes and DON induced anorexia, including calcium sensing receptor (CaSR) which produces satiety hormones and activates parathormone synthesis.

Key words: deoxynivalenol, calcium, antioxidant, oxidative stress, vitamin D, bone mineralization

Effect of Citral on Endotoxin-induced Oxidative Stress in Gut Epithelial Cells

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Abstract

Maintaining a healthy gut is important in animal production as it directly affects nutrient utilization. Gastrointestinal infections can lead to oxidative stress in the gut and impair production performances.. Citral, an essential oil, has recently received attention as a potential antibiotic alternative to be used in monogastric animals to control pathogenic infections. Lipopolysaccharides (LPS), an endotoxin produced by gram-negative bacteria is used to induce oxidative stress in gut epithelial cells (Caco-2 cells), folllowing the preincubation with citral. Biochemical assays were used to measure oxidative stress related parameters while the t-test was used in statistical analysis. Lipopolysaccharides caused a significant increased in oxidative stress biomarker lipid peroxidation (MDA) and a reduction of endogenous antioxidants (SOD and GSH). Such an effect was time and dose dependent. Preincubation of cells with citral (25 µM) significantly attenuated LPS-induced lipid peroxidation and restored SOD and GSH levels. These results have demonstrated that citral has a protective effect on LPS-mediated oxidative stress in gut epithelial cells through the down regulation of lipid peroxidation and improving the endogenous antioxidant system. Supplementation of citral in feed may exert a beneficial effect on oxidative stress-induced gut injury during infection.

Keywords: animals, citral, gut health, lipopolysaccharides, oxidative stress

Sorting Efficiency and Grain Recovery from Deoxynivalenol Contaminated Wheat by Near-Infrared Transmittance Sorting (Bomill) is Impacted by Calibration Type and HVK Setting

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Abstract

Fusarium infection of wheat causes the production of the secondary metabolite deoxynivalenol (DON), which affects animal performance. Limited post-harvest sorting technologies are available to remove moderately infected kernels and improve overall grain recovery below 5ppm DON. Proprietary near-infrared seed sorting calibrations for *fusarium* damage have recently been developed by BoMill AB (Sweden) to achieve this objective. Two experiments were conducted to test the sorting efficacy of the BoMill IQ using the new calibrations. Wheat with 8ppm DON was pre-cleaned to remove debris and small shriveled kernels prior to sorting. The impact of HVK setting and calibration (*fusarium* and protein) was tested. Recovery of low *fusarium* grain using the *fusarium* calibration settings was 43.4, 50.3, and 44.9%, with rejection rates of 38.7, 29.0, and 22.7%, and average DON in recoverable fractions was 2.0, 1.7, and 2.4 ppm, using the HVK, HHVK, and HHHVK settings respectively. Using the protein calibration setting, grain recoveries were 46.2 and 51.1%, with rejection rates of 35.6, and 17.5%, and average DON in recoverable fractions was 1.6, and 3.0 ppm, using the HVK, and HHVK settings respectively. Therefore, it was determined the *fusarium* calibration using the HHVK setting was most effective, as it resulted in lower average DON with lower rejection rates. The second experiment examined sorting efficiency using the optimal setting on a pooled 6 ppm wheat, and a 15 ppm wheat. Grain recovery from the pooled and 15 ppm wheats were 69.0 and 46.1%, with an average DON of 1.3, and 2.2 ppm respectively. Sorting using a BoMill equipped with the fusarium calibration and HHVK setting will effectively sort a range of wheat samples, including pooled samples, into low DON fractions, increasing the grain value and minimizing any negative effects on the animal.

Key words: BoMill, DON, grain recovery, near-infrared transmittance, sorting, wheat

Sorting of Heavily *Fusarium* Infected Wheat Using a Combination of Air Fractionation and Near-Infrared Transmittance (BoMill) to Improve Grain Recovery with Low DON Levels

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Abstract

Fusarium infection of wheat causes the production of the secondary metabolite deoxynivalenol (DON), which is toxic to animals. Sorting technologies such as air fractionators and optical sorting have limited ability to separate mildly infected grain from uninfected material. Air fractionation (AF) sorts based on the relative grain density, while Near-Infrared Transmittance (NIT) sorts based on the relative chemical composition. We hypothesised that using a combination of AF followed by NIT sorting would increase efficiency and grain recovery while reducing DON. Three wheats were processed through a GCS-200 Grain Cleaner (Apple Valley, MN), fan setting 33.5Hz, into five fractions. The DON levels of the wheats were 8ppm (W1), 15ppm (W2), and 6ppm pooled wheat (W3) (15kg wheat x 4 reps). All fractions were weighed, and DON concentration determined by ELISA. The third fraction had moderately high DON (9 ppm) and was further sorted using a BoMill IQ (BoMill AB, Sweden) with the HHVK setting in the *Fusarium* calibration. Each of the seven fractions were weighed, and DON concentration measured. AF grain recovery <5 ppm was 52.5%, 42.5%, and 59.2% for W1, W2, and W3 respectively. The BoMill IQ grain recovery results were used from a previous experiment, 50.5% (W1), 46.1% (W2), and 69% (W3; W3 required two different sized sorting discs and had to be separated by kernel size). By using AF and the BoMill IQ sequentially, the grain recovery increased to 71.5% for W1, 56.6% for W2, and 72.3% for W3. Sequential sorting using AF and the BoMill is an effective method to recover significant quantities of low DON wheat from high DON wheat. AF acts as a grain pre-cleaner to remove chaff and debris, and separates kernels by size, thereby increasing the sorting efficiency of the BoMill and processing capability, which can reduce the necessity of grain blending for DON reduction.

Key words: air fractionation, BoMill, DON, sequential sorting, wheat

Intestinal delivery of micro-encapsulated zinc oxide to manage post-weaning diarrhea in piglets

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Abstract

The post-weaning period marks a two-week window that is a vulnerable time for piglets. During this period, they are at an increased risk for illness – particularly diarrhea, which can lead to a failure to thrive, and in the most severe cases, death. The cause of diarrhea is often due to *Escherichia coli* intestinal infection and interventions typically includes antibiotic use. However, because of the push to be less antibiotic-dependent, non-drug alternatives have gained more favour. One of these options include zinc oxide (ZnO), an inorganic compound found to have antibacterial properties. Its integrative use in the animal industry has already suggested its efficacy in managing post-weaning diarrhea. However, it has been subject to overuse which has lead to major downstream polluting effects and animal toxicity. Therefore, research advances are in effort to reduce the amount of ZnO delivered to piglets while maintaining a potent effect against *E. coli*. This research evaluates the encapsulation of ZnO within natural alginate and whey protein or a commercially purchased polymer, Eudragit L100, in order to achieve this goal. The purpose of the encapsulating ingredients is for the targeted delivery of ZnO to the infected intestinal location, allowing ZnO to act with specificity and in a concentrated dose. It was found that through *in vitro* studies using porcine digestive fluids, encapsulation of ZnO within Eudragit L100 was capable of delivering 70% of the total encapsulated ZnO to the intestinal region, while only 50% was delivered when ZnO was encapsulated in alginate and whey protein. The ability of the encapsulation materials to protect against pre-mature release of ZnO during gastric transit can account for the difference in these results. Therefore, ZnO encapsulation within Eudragit L100 demonstrates greater prospect for a complete delivery of ZnO to the intestinal region with minimal loss. The broader implications of this suggest a possible solution to reduce the overuse of ZnO, while preserving its effective interventional use in the animal industry.

Key words: post-weaning diarrhea, zinc oxide, encapsulation

Effects of Encapsulated Cinnamaldehyde and Citral on Performance and Cecal Microbiota of Broilers Vaccinated or not Vaccinated against Coccidiosis

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Abstract

This study investigated the effects of encapsulated cinnamaldehyde (CIN) and citral (CIT) alone or in combination (CIN + CIT) on the growth performance and cecal microbiota of nonvaccinated broilers and broilers vaccinated against coccidiosis. Vaccinated (1,600) and nonvaccinated (1,600) 0-day-old male Cobb500 broilers were randomly allocated to 5 treatments: basal diet (control) and basal diet supplemented with bacitracin (BAC, 55 ppm), CIN, CIT, and CIN + CIT at 100 ppm each. In general, body weight (BW) and feed conversion ratio (FCR) were significantly improved in birds treated with BAC, CIN, CIT, and CIN + CIT but were all decreased in vaccinated birds compared with nonvaccinated birds. Significant interactions between vaccination and treatments for average daily gain during the periods of starter (day 0–9) and BW on day 10 were noted. Broilers receiving vaccines or feed with BAC, CIN, CIT, or CIN + CIT showed significant reductions in mortality rate from day 0 to 28. The incidences of minor coccidiosis were higher in vaccinated birds than in nonvaccinated birds ($P <$ 0.05). Diet with BAC or tested encapsulated essential oils showed comparable effects on the coccidiosis incidences. Similar to BAC, CIN and its combination with CIT reduced both incidence and severity of necrotic enteritis ($P <$ 0.05). No treatment effects were observed on the cecal microbiota at the phyla level. At the genus level, *Lactobacillus* was more abundant in broilers fed with CIT, while *Clostridium* and *Enterococcus* were less abundant in broilers fed with CIN, CIT, or CIN + CIT in both the vaccinated and nonvaccinated groups. Results from this study suggested that addition of CIN alone or in combination with CIT in feed could improve chicken growth performance to the level comparable with BAC and alter cecal microbiota composition.

Key words: broiler, ceca microbiota, coccidiosis, essential oils

Novel nanofiber mats for effective delivery of sodium metabisulfite (SMBS) to the intestinal tract of pigs

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Abstract

Deoxynivalnol (DON) occurs on many commonly used cereal grains. DON levels as low as 0.6 to 2.0 ppm in complete feed cause a reduction in feed intake and growth rate, damage to the intestinal epithelial cells and increased susceptibility to enteric pathogen challenge. Sodium metabisulfite (SMBS) is a sulfite reducing agent commonly added to animal feeds to improve protein digestibility by cleaving disulfide bonds. It has been shown that SMBS can destroy 70%-100% of DON in processed grains or feeds *in vitro* with 0.45%-0.9% levels at pH around 6.5 but not at acidic conditions. SMBS can be degraded quickly under aqueous acid conditions such as pig stomach to form sulfur dioxide and subsequently decompose into sodium oxide and sulfur dioxide. Therefore, little SMBS will remain intact in the small intestine where an optimal pH environment exists for SMBS to detoxify DON. Thus, the aim of this study is to encapsulate SMBS into nanofiber mats by an electrospinning technology to deliver intact SMBS to the lower gut such as the small intestine to detoxify DON effectively. Electrospinning was implemented to prepare nanofiberous mats from a suspension of particulate SMBS in the solution of a pH sensitive polymer (Eudragit L100-55). The highest loading capacity and loading efficiency of SMBS achieved in the nanofiberous mats were 32.00% and 80.01%, respectively. *In vitro* release studies showed that 49.17% of encapsulated SMBS was released in the simulated gastric fluid (SGF) in 2 h and 50.83% of SMBS release was observed in the simulated intestinal fluid (SIF) within 2 h. Additionally, in an *in vitro* DON detoxification experiment using IPEC-J2 cells, the ability of DON detoxification in DON (1 μg/mL) and nanofiberous mat with 1% SMBS group was reflected by 82.99% cell viability. For the first time, we demonstrate successful encapsulation of particulate SMBS in a nano-vehicle (electrospun nanofiber), achieve >50% release of SMBS in SIF, and effective detoxification of DON in an *in vitro* cell assay.

Key words: sodium metabisulfite (SMBS), deoxynivalenol (DON), nanofiber, release, pig

In Vitro Characterization of Pig Calcium-Sensing Receptor (CaSR) Ligands and Cell Signaling Pathways Related to CaSR Activation using a Dual-Luciferase Reporter Assay

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Abstract

Our previous study demonstrated that pig calcium-sensing receptor (pCaSR) is widely expressed in different tissues including intestinal segments in weaned piglets and the ileum has a higher expression level of pCaSR. Further research is needed to characterize the functions of pCaSR, its potential ligands and cell signaling pathways related to CaSR activation in enteroendocrine cells and potentially in enterocytes. In the present study, a dual-luciferase reporter assay was developed to investigate the potential ligands of pCaSR and the ligand-mediated signaling pathways. The results showed that extracellular calcium activated pCaSR in a concentration-dependent manner with $EC_{50} = 4.27$ mM through the Gq/11 signaling pathway, $EC_{50} = 2.76$ mM through Gs signaling, $EC_{50} = 2.85$ mM through ERK1/2 activation signaling, and $EC_{50} = 2.26$ mM through RhoA activation signaling. Given that Gs signaling, ERK1/2 activation signaling and RhoA activation signaling pathways shared similar EC_{50} values by the extracellular calcium stimulation, it was possible that the activation of pCaSR might lead to changes in cAMP production, ERK1/2 and RhoA activation with similar efficacies. In contrast, EC_{50} value through Gq/11 signaling was more than 1.5 times higher than those by the other three signaling pathways tested, indicating that pCaSR increases intracellular calcium concentration less efficiently. Meanwhile, compared with the control group, the pCaSR activation response triggered by α-casein was significantly higher in all four reporter assays ($P < 0.05$), which demonstrated that α-casein was an agonist for pCaSR. Moreover, it implied that α-casein could active pCaSR through four main signaling pathways. In conclusion, similar to the human CaSR, pCaSR also showed biased agonism through four main signaling pathways. Further studies are needed to investigate the agonist type of α-casein and related potencies.

Key words: calcium-sensing receptor, pigs, α-casein, agonist, ligand

Contrasting Standard and Low Lignin Alfalfa in Mixtures with Grasses

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Abstract

This study aimed at comparing the performance of standard and non-GM low lignin alfalfa on forage yield, botanical composition, chemical composition, alfalfa persistence and in vitro total tract NDF digestibility (TTNDFD) when mixed with cool-season perennial grasses. Treatments included standard or low lignin alfalfa (13 kg/ha) seeded in mixtures with tall fescue or timothy (5 and 8 kg/ha, respectively), which were assigned to randomized complete block design with four replications. Experimental plots were seeded in May and were harvested at either the early bud or early flower stages of development of alfalfa for two production years. Forage samples from each plot were chemically analyzed for NDF, ADF, ADL and CP. Alfalfa winter survival were determined by measuring alfalfa stem density in the fall and spring of the following year. Data were analyzed separately for each year using the PROC MIXED procedure of SAS. In the first production year, total forage yield did not differ between treatments. However, in the second year, total yields were higher when both alfalfa types were mixed with tall fescue compared to timothy. Alfalfa yields were higher when mixed with timothy in year one but higher with tall fescue in year two. In both years, yield of tall fescue was higher than that of timothy for both types of alfalfa. We are currently finalizing our chemical analyses and in vitro digestibility analyses (24, 30, 48 and 240h) for TTNDFD determination.

Key words: alfalfa, low lignin, in vitro digestibility, winter survival

Feeding Garlic Powder at Low Doses Does Not Negatively Affect Feedlot Cattle Health

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Abstract

Antimicrobial and insectifugal effects have been reported for dehydrated garlic powder. With limited knowledge on its health impact on beef cattle, this study investigated whether free-choice summer supplementation infused with garlic powder affected cattle blood parameters and long-term stress during the summer. A total of 104 crossbred steers in feedlot setting were randomly assigned to either 0% garlic product (Control), 0.3% garlic-oil-based premix (GOil), 2.5% garlic powder (2.5% GP) or 5% garlic powder (5% GP). Feed and mineral supplements were fed separately in individual GrowSafe bunks to monitor individual mineral consumption. Animal performance measures collected include body weights and gains, individual feed intake, measures of body composition, blood biochemical and hematological parameters. Hair samples were clipped from each steer (3 times) from the rump region to evaluate differences cortisol levels as a measure of long-term stress. Preliminary results showed no difference in final body weight (P=0.19) and dry matter intake (P=0.89). However, compared to the control, all garlic treatments significantly increased daily mineral intake (P=0.003). In addition, GOil had the highest average daily gain (P=0.002) and the highest gain to feed ratio (P=0.01). No significant difference (P>0.05) was observed in most serum biochemical parameters with the exception of 5% GP that had a significantly lower bilirubin (P=0.0001) and albumin (P=0.002) concentration but all biochemical and hematological measurements fell within the normal range for beef cattle. There was no difference (P=0.29) among the groups for cortisol levels. The preliminary results show that low-dose infusion of garlic powder was not associated with any adverse health conditions and may be used to increase mineral consumption in cattle.

Key words: garlic powder, additive, performance, health

Effects of subacute ruminal acidosis on dry matter intake, rumen fermentation, blood variables, and conception rate in Holstein heifers

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Abstract

The objective of this study was to determine if experimentally induced subacute ruminal acidosis (SARA) reduces conception rates of Holstein heifers. One hundred and ten heifers were synchronized for artificial insemination by two injections of PGF2α in a 13-d interval and assigned randomly to two treatments. The control heifers received a diet containing 32% (DM basis) barley-based concentrate, while the SARA challenge group received a diet containing 68% of this concentrate started 3 d after the 2nd PGF2α injection and continued for seven days. Forty-one heifers from the SARA group and 39 heifers from the control group showed visible signs of heat and were inseminated. Heifers in SARA group had higher dry matter intake (DMI, 10.4 vs. 9.0 kg d^{-1}, $P < 0.01$) and lower rumen pH (6.02 vs. 6.45, $P < 0.01$) and fecal pH (6.71 vs. 6.97, $P < 0.01$). The SARA challenge increased rumen concentrations of lipopolysaccharide, lactate, propionate and valerate, but did not affect the concentrations of acetate, and butyrate. The challenge did not affect glucose, urea nitrogen, aspartate aminotransferase, calcium and cortisol concentrations in blood, but it lowered blood betahydroxy butyrate ($P < 0.01$). Induction of SARA markedly reduced first service conception rate tested by ultrasonography 28 d after insemination (53.7 vs. 71.8 %, $P < 0.05$). Additionally, 100% of control heifers that were confirmed as pregnant in the 28-d test were also pregnant at 60-d test, whereas this ratio was only 73.9% ($P < 0.01$) in SARA-challenged heifers suggesting that SARA had a persistent effect on reproduction. Results suggest negative effects of SARA on fertility of dairy heifers.

Key words: metabolic acidosis, Fertility, Heifers, Conception rate

Evaluating the Establishment of Perennial Forages with Warm-Season Annual Companion Hybrids on Forage Yield and Quality

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Abstract

The objective of this study was to evaluate the effects of perennial mixtures (alfalfa, clover and tall fescue; control) seeded alone or with a companion forage [Sudangrass (SG), Sudangrass brown midrib (BMR) gene 12 (BSG), Sorghum-Sudangrass BMR gene 6 (BSSG) or oat] on forage yield, chemical composition and in vitro total tract NDF digestibility (TTNDFD]. Experimental plots (8 replicates/treatment) were harvested 60 d (1st cut) and 90 d (2nd cut) at bud stage of alfalfa. The TTNDFD was estimated from potentially digestible NDF (pdNDF), digestion rate and passage rate while forage indigestible NDF (iNDF) was calculated from 240 h in vitro incubation. Potentially degradable NDF was calculated by subtracting iNDF from total NDF whereas digestion rate was estimated by in vitro incubation at 24, 30 and 48 h. Data were analyzed as repeated measures using the MIXED procedure of SAS with fixed effects of treatment and cut. Results showed that total forage yield (2nd cut) was higher ($P < 0.0001$) for SG (6.8 T/ha), BSSG (6.7 T/ha) and BSG (6.3 T/ha) than control (4.1 T/ha). Oat produced 87% less forage yield in the second cut than first cut. Companion forages reduced ($P < 0.0001$) the yield of perennial legumes and weeds. Relative to control, companion forages reduced ($P < 0.0001$) lignin and crude protein but increased ($P < 0.0001$) NDF and ADF contents of harvested forages. Water-soluble carbohydrate concentration was highest with oat (12.4%), intermediate with SG (9.5%), BSG (9.1%) and BSSG (10.7%) and lowest with control (7.0%). Estimated milk yield was higher ($P < 0.0001$) for companion forages than control. The iNDF was lower ($P < 0.0001$) with BSSG than control and oat treatment. In vitro TTNDFD of second cut followed the order ($P < 0.0001$): BSG and BSSG (average 62.2%) > SG (58.5%) > oat (53.3%) > control (52.3%). It was concluded that establishing perennial forages with SG, BSG or BSSG may improve forage yields, nutritive value and fiber digestibility.

Key words: cows, forages, fiber digestibility

Evaluating Silage Quality of Perennial Forages Established with Warm-Season Annual Companion Crops

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Abstract

This study was conducted to evaluate the effects of perennial forage mixture (alfalfa, clover and tall fescue; control) seeded in a pure stand or with different annual companion forages [Sudangrass (SG), Sudangrass brown midrib (BMR) gene 12 (BSG) or Sorghum-Sudangrass BMR gene 6 (BSSG)] on ensiling characteristics, chemical composition and in vitro total-tract NDF digestibility (TTNDFD) of experimental silages. Field experimental plots (8 per treatment) were harvested at 60 d (1st cut) and 90 d (2nd cut) at the bud stage of alfalfa. Second harvest forages were ensiled in laboratory silos for 42 days. Silage indigestible NDF (iNDF) was calculated by in vitro incubation at 240 h whereas potentially degradable NDF (pdNDF) was calculated by subtracting iNDF from total NDF. Digestion rate (kd) of pdNDF was estimated by in vitro incubation at 24, 30 and 48 h. Data were analyzed as repeated measures using the MIXED procedure of SAS with fixed effects of treatment and cut. Chemical analysis of experimental silages showed that companion forages reduced lignin ($P < 0.0001$) and crude protein ($P = 0.0006$) concentrations but increased NDF and ADF contents ($P < 0.0001$) compared with the control. Neutral detergent insoluble protein, water soluble carbohydrates, total digestible nutrients, and net energy of lactation were similar across treatments whereas acid detergent insoluble protein was higher ($P < 0.0001$) for control than other treatments. Indigestible NDF fractions were lower ($P = 0.002$) in warm-season companion forages (average 19.5%) than control (28.9%). Digestion rate of pdNDF was higher ($P < 0.0001$) in SG, BSG and BSSG than control. In vitro TTNDFD followed the order ($P < 0.0001$): BSG and BSSG (average 64.7%) > SG (62.8%) > control (56.4%). It was concluded that seeding perennial forages with SG, BSG or BSSG may improve chemical composition and NDF digestibility of silages.

Key words: dairy cows, silage, fiber digestibility

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