ANCC

2017 ANIMAL NUTRITION CONFERENCE OF CANADA

CNAC

2017 COLLOQUE DE NUTRITION ANIMALE DU CANADA

Proceedings

Cahier de conférences

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May/mai 2017

Quebec city

Ville de Québec

Nutritional Strategies to Reduce the Use of Antimicrobials in Animal Production

Stratégies nutritionnelles visant à réduire l'usage des antimicrobiens dans la production animale

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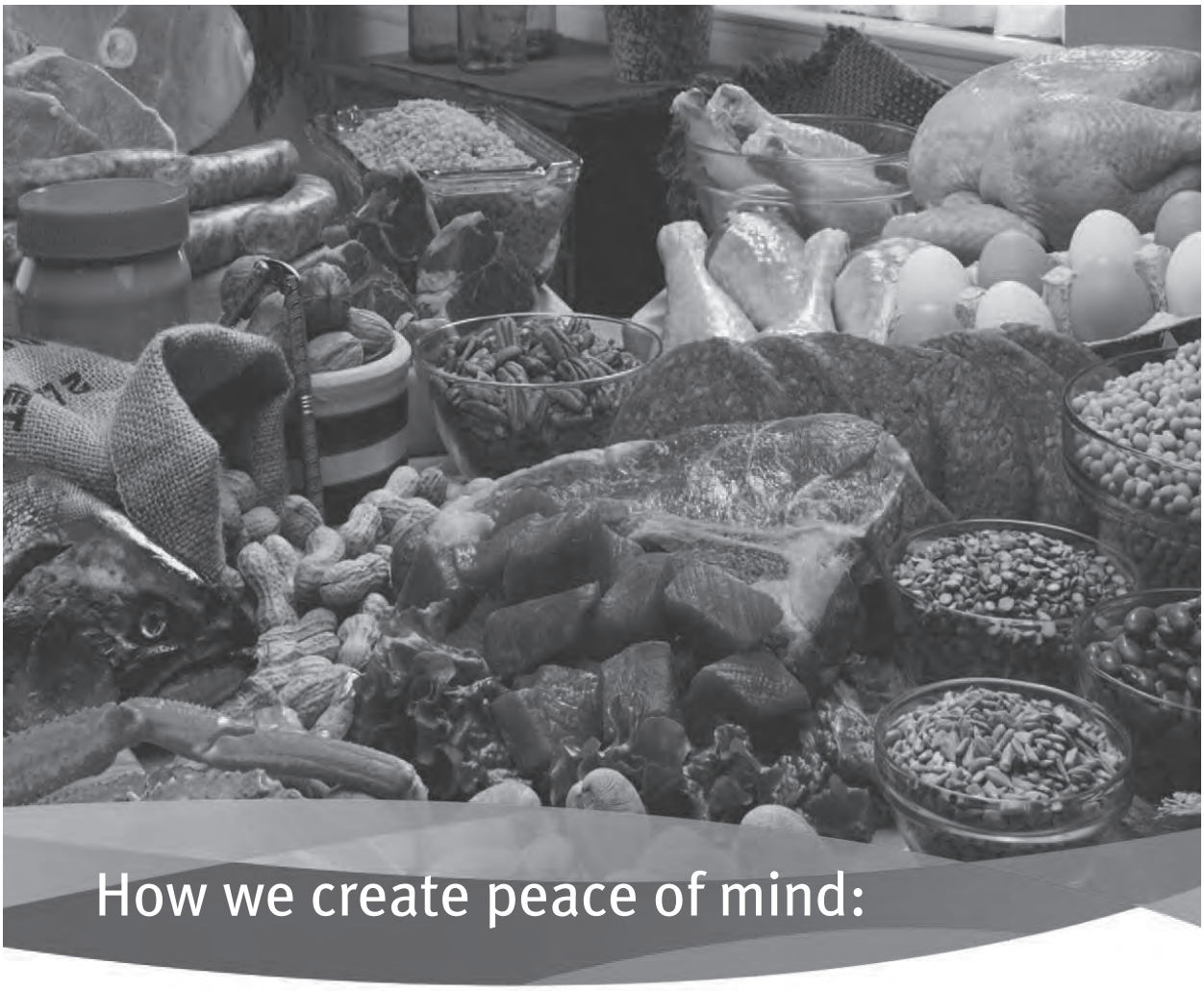

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Welcome from ANAC / *Bienvenue de l'ANAC*

The Animal Nutrition Association of Canada (ANAC) is pleased to welcome you to the inaugural edition of the Animal Nutrition Conference of Canada (ANCC). As the demand for protein increases and the feed industry faces regulatory changes and new consumer demands, it is important to have a national platform where new technologies and management practices can be shared and discussed. This world-class event provides learning and networking opportunities for the animal nutrition community across Canada and the world. We invite you to innovate, grow, and connect.

The conference organizing committee has planned an exceptional program and ANAC would like to thank them for their dedication to help ensure the first ANCC is a success. We would also like to thank our presenters whom are sharing their innovative research and knowledge. This conference would not have been possible without the generous sponsorship from our industry partners.

We hope you enjoy the conference and look forward to welcoming you in future years.

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

L'Association de nutrition animale du Canada (ANAC) est heureuse de vous accueillir à la première édition du Colloque de nutrition animale du Canada (CNAC). À mesure que la demande de protéines augmente et que l'industrie de l'alimentation du bétail est confrontée à des changements réglementaires et aux nouvelles demandes des consommateurs, il devient important de disposer d'une plateforme permettant de discuter des nouvelles technologies et de partager les connaissances et les pratiques de gestion. Cet événement de classe mondiale offre des possibilités d'apprentissage et de réseautage pour tous les intervenants de la nutrition animale provenant de l'ensemble du Canada, et même du monde entier. Il s'agit d'une invitation à innover, à grandir et à partager.

Le comité organisateur du colloque a planifié un programme exceptionnel et l'ANAC tient à le remercier pour le dévouement à faire du premier CNAC une vraie réussite. Nous tenons en outre à remercier nos présentateurs qui ont décidé de partager leurs recherches innovantes et leurs connaissances. La tenue de ce colloque n'aurait pas été possible sans les généreuses commandites de nos partenaires de l'industrie.

Nous espérons sincèrement que vous aimerez ce colloque et nous nous réjouissons à l'idée de vous accueillir de nouveau au cours des prochaines années.

Melissa Dumont, agronome
Directrice exécutive, Association de nutrition animale du Canada

Welcome from the ANCC Organizing Committee 2017
Bienvenue du *Comité organisateur du CNAC 2017*

Dear friends and industry members,
It is an honour to welcome you to this 1st edition of the Animal Nutrition Conference of Canada. The conference's organizing committee, made up of several active members of industry and of the scientific community, chose to focus on nutritional strategies to reduce the use of antimicrobials in animal production. Apart from the new legislative constraints that will force the industry to adapt and find new ways to maintain livestock profitability, there is also an opportunity for us to better understand the nutritional aspects involved in maintaining health and performance of our herds. The committee has sought to bring together different experts in Canada and elsewhere to present the state of our knowledge from several angles. This includes research findings from some of Canada's best and brightest students. You can also learn more about a few of the industry's recent innovations at the supplier showcase. The ultimate goal is to continuously improve the productivity and profitability of our industry while meeting the expectations of consumers.

Chers amis et membres de l'industrie,

C'est un honneur de vous accueillir pour cette 1re édition du Colloque de Nutrition animale du Canada. Le comité organisateur de cette conférence, composé de plusieurs membres actifs de l'industrie et du domaine scientifique, a choisi de cibler sur les stratégies nutritionnelles pour réduire l'utilisation des antimicrobiens. Mis à part les nouvelles contraintes législatives qui forceront l'industrie à s'adapter et trouver des moyens de maintenir la profitabilité des élevages, il y a aussi une opportunité pour mieux comprendre les aspects nutritionnels qui sont en cause dans le maintien de la santé et la performance de nos troupeaux. Le comité s'est efforcé de regrouper différents experts au Canada et d'ailleurs, pour présenter l'état de nos connaissances sous plusieurs angles. Ceci inclut les résultats de recherche de certains des meilleurs étudiants du Canada. Vous pouvez également en apprendre plus sur certaines innovations récentes de l'industrie au salon des fournisseurs. L'objectif ultime étant d'améliorer sans cesse la productivité et la rentabilité de notre industrie tout en répondant aux attentes des consommateurs.

Christian Bruneau
Industry Co-Chair
Co-président, de l'industrie
Cargill Animal Nutrition

Rachel Gervais
Academic Co-Chair
Co-présidente, de l'académie
Université Laval

Raylene Boehmer
Hi-Pro Feeds LP

My-Lien Bosch
Animal Nutrition Association of Canada
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Shawn Fairbairn
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2017 ANCC CONFERENCE PROGRAM
PROGRAMME DU COLLOQUE CNAC 2017

May 10-11, 2017 – Hilton, Quebec City, Quebec
Les 10 et 11 mai, 2017 – Hilton, Québec (QC)

Nutritional strategies to reduce antimicrobial usage in animal production

Stratégies nutritionnelles pour réduire l'utilisation des antimicrobiens en production animale

WEDNESDAY, MAY 10TH – MORNING / *MERCREDI LE 10 MAI – MATIN*

Pre-Conference Symposium
Symposium précolloque
Room/*Salle*: Palais

Antibiotic-Free Livestock and Poultry Production: Challenges and Solutions
La production de bétail et de volaille sans antibiotiques : défis et solutions

7:00	Registration and breakfast *Inscriptions et petit déjeuner*	
8:00	Overview of ABF livestock and poultry production *Survol de la production sans antibiotiques de bétail et de volaille*	**Dr. Raj Murugesan** BIOMIN America, Inc., USA
8:15	Nutritional requirement on the immune function of livestock species *Besoins nutritionnels pour la fonction immunitaire des animaux d'élevage*	**Dr. Lance Baumgard,** Iowa State University
9:00	Nutritional and management challenges and solutions in ABF swine production *Nutrition et gestion en production porcine sans antibiotiques : défis et solutions*	**Dr. Laura Greiner** Carthage Innovative Swine Solutions, LLC, Illinois
9:45	Break / *Pause* Room/*Salle*: Foyer	
10:00	A nutritionist's perspective on the antibiotic removal in poultry production *Perspective d'un spécialiste en nutrition animale quant au retrait des antibiotiques en production avicole*	**Dr. Shivaram Rao** Pilgrims Pride, USA
10 :45	Raising healthy birds without antibiotics *Élever des oiseaux sains sans recours aux antibiotiques*	**Dr. Scott Gustin** Tyson Foods, USA
11:30	Panel discussion and Q&A *Panel de discussion et Q et R*	All speakers / *Tous les conférenciers*
12:00	Lunch / *Dîner* Room/*Salle*: St. Louis/Kent	

**Sessions marked with asterisks will be presented in French. All other sessions will be presented in English.
***Les séances marquées avec astérisque seront présentées en français. Toutes les autres séances seront présentées en anglais.*

WEDNESDAY, MAY 10TH – AFTERNOON / *MERCREDI LE 10 MAI – APRÈS-MIDI*

Conference registration begins at 7 am
Inscriptions pour le Colloque commence à partir de 7h00

Opening Plenary / *Plénière d'ouverture* Room/*Salle*: Palais		
1:00	ANAC welcome *Mot de bienvenue de l'ANAC*	**Melissa Dumont** ANAC Executive Director *Directrice exécutive de l'ANAC*
1 :05	Welcome and opening remarks *Mot de bienvenue et propos d'ouverture*	**Christian Bruneau** Conference Co-Chair *Co-Président du Colloque*
1:10	Antimicrobial consumption and resistance – a One Health view *Consommation d'antimicrobiens et résistance – optique de One Health*	**Dr. Ramanan Laxminarayan** Center for Disease, Dynamics, Economics & Policy (CDDEP) & Princeton University
1:55	New regulations and policies in Canada related to antibiotic usage and impacts on feed *Nouveaux règlements et politiques sur l'utilisation des antibiotiques au Canada*	**Dr. Mary Jane Ireland** Veterinary Drugs Directorate, Health Canada
2 :40	Break / *Pause* Room/*Salle*: Foyer	
3:10	The end of antimicrobials: A biological reality or a consumer choice? *La fin des antimicrobiens : réalité biologique ou choix des consommateurs?*	**Dr. Tim McAllister** Agriculture and Agri-Food Canada (AAFC)
3:55	The three levels of immunity in the animal: A case of a good defense preventing too much offense (and damage) *Les trois niveaux d'immunité dans l'animal : le cas d'une bonne défensive évitant trop d'offensive (et de dommages)*	**Dr. Chris Chase** South Dakota State University
4:40	ANAC scholarship announcement and recipient's presentation *Annonce de la bourse d'études de l'ANAC et présentation du récipiendaire*	**Haley Leung** University of Guelph Lauréate*/Recipient*
5 :00	End of Opening Plenary *Fin de la plénière d'ouverture*	
5:00 - 7:30	**ANCC 2017 Reception:** Come and enjoy an evening of food (cocktail dinner), drinks and networking around the supplier showcase and graduate student poster competition. ***Réception du CNAC 2017 :*** *Venez profiter d'une soirée où l'on servira nourriture et boissons (cocktail dînatoire), alors que vous aurez l'opportunité de réseauter autour de la vitrine d'exposition des fournisseurs et du Concours d'affiches pour étudiants diplômés.* Room/*Salle*: Foyer	

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THURSDAY, MAY 11TH – MORNING / *JEUDI LE 11 MAI – MATIN*

7 am Hot breakfast / *7h00 Petit déjeuner chaud*
Room/*Salle*: St. Louis/Kent

Concurrent Sessions / *Séances concurrentes*

Monogastric Session / *Séance sur les monogastriques* Room/*Salle*: St. Louis/Kent		
8:00	Measuring the effects of early life perturbations on immune development in pigs and poultry *Mesure des effets des perturbations en bas âge du développement immunitaire des porcs et des volailles*	**Dr. Dirkjan Schokker** Wageningen University & Research Centre, The Netherlands
8:45	Controlling coccidiosis in poultry fed diets free of anti-cocci and anti-microbial products *Contrôle de la coccidiose chez la volaille nourrie d'aliments sans produits anticoccidiose et antimicrobiens*	**Dr. Brett Lumpkins** Southern Poultry Research, Georgia
9 :30	Managing gut function and health in non-ruminants without antimicrobials: The role of nutrition *Rôle de la nutrition dans la gestion de la santé et des fonctions intestinales des monogastriques élevés sans antimicrobiens*	**Dr. Elijah Kiarie** University of Guelph
10 :15	Break / *Pause* Room/*Salle*: Foyer	
10 :30	Growth performance and indices of gut function and health in broiler chickens fed corn-soybean meal diets without or with exogenous epidermal growth factor upon challenge with Eimeria. *Rendement de croissance et indices de fonction intestinale et de santé chez les poulets à griller nourris au maïs et au tourteau de soja, sans et avec facteur de croissance épidermique exogène en présence d'Eimeria*	**Emily Kim** Graduate student, University of Guelph
10 :45	Exploring modes of action and efficacy for alternative gut health modulating additives in young pigs *Examen des modes d'action et de l'efficacité des additifs de rechange de modulation de la santé intestinale chez les jeunes porcs*	**Dr. John Pluske** Murdoch University, Australia
11:30	Immuno-Epigenetics: A veterinarian's perspective on reducing the use of antimicrobials through the use of nutritional immunology *Immuno-épigénétique : la perspective d'un vétérinaire dans la réduction de l'utilisation des antimicrobiens par l'utilisation de l'immunologie nutritionnelle*	**Dr. Juan Carlos Rodriguez-Lecompte** University of Prince Edward Island
12:15	Lunch / Dîner Room/*Salle*: St. Louis/Kent	

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THURSDAY, MAY 11TH – MORNING / *JEUDI LE 11 MAI – MATIN*

7 am Hot breakfast / *7h00 Petit déjeuner chaud*
Room/*Salle*: St. Louis/Kent

Concurrent Sessions / *Séances concurrentes*

Ruminant Session / *Séance sur les ruminants* Room/*Salle*: Palais		
8:00	Bovine immunity and strategies to reduce health disorders of the transition cow *Immunité bovine et stratégies pour réduire les troubles de santé chez les vaches en transition*	**Dr. Lorraine Sordillo** Michigan State University
8:45	Dairy cow ketosis: novel biomarkers for early detection of disease risk *Cétose de la vache laitière : nouveaux biomarqueurs servant à la détection précoce de risques de maladie*	**Dr. Burim Ametaj** University of Alberta
9:30	Challenges of identifying viable alternatives to antimicrobials in beef cattle production *Défis d'identification de solutions de rechange viables aux antimicrobiens en production bovine*	**Dr. Tim McAllister** Agriculture and Agri-Food Canada (AAFC)
10:15	Pause / *Break* Room/*Salle*: Foyer	
10:30	*** Évaluation de la variabilité du transfert d'immunité passive dans les troupeaux laitiers du Québec* Evaluating the variability of passive immunity transfer in Quebec dairy herds	**Marie-Pascale Morin** Étudiante diplômée, Université de Montréal
10:45	Leaky gut's contribution to inefficient nutrient partitioning *Contribution de l'intestin perméable à l'inefficacité d'utilisation des nutriments*	**Dr. Lance Baumgard** Iowa State University
11:30	Impact of transition health status of dairy cows on reproductive performance: Looking at potential nutritional tools *Impact de l'état de santé des vaches laitières en transition sur la performance reproductrice : recherche d'outils nutritionnels*	**Dr. Ronaldo Cerri** University of British Columbia
12:15	Lunch / *Dîner* Room/*Salle*: St. Louis/Kent	

THURSDAY, MAY 11TH – AFTERNOON / *JEUDI LE 11 MAI – APRÈS-MIDI*

Closing Plenary / *Plénière de cloture* Room/*Salle*: Palais		
1:15	***Améliorer la santé et la résistance des animaux en production porcine et laitière par l'utilisation d'une stratégie nutritionnelle novatrice ciblant la mitochondrie* Improving health and resistance of animals in swine and dairy production by using an innovative nutritional strategy targeting mitochondria	**Dr. Jérôme Lapointe** Agriculture et Agroalimentaire Canada
2 :00	Consumers' Attitudes on Meat Production: What They Know, What They Don't Know and Why It Matters *Attitudes des consommateurs face à la production de viande : ce qu'ils savent, ne savent pas et pourquoi cela importe*	**Dr. Mike von Massow** University of Guelph
2:45	Closing remarks / *Propos de clôture*	**Rachel Gervais** Conference Co-Chair *Co-Présidente du Colloque*

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Pre-Conference Symposium
Symposium précolloque

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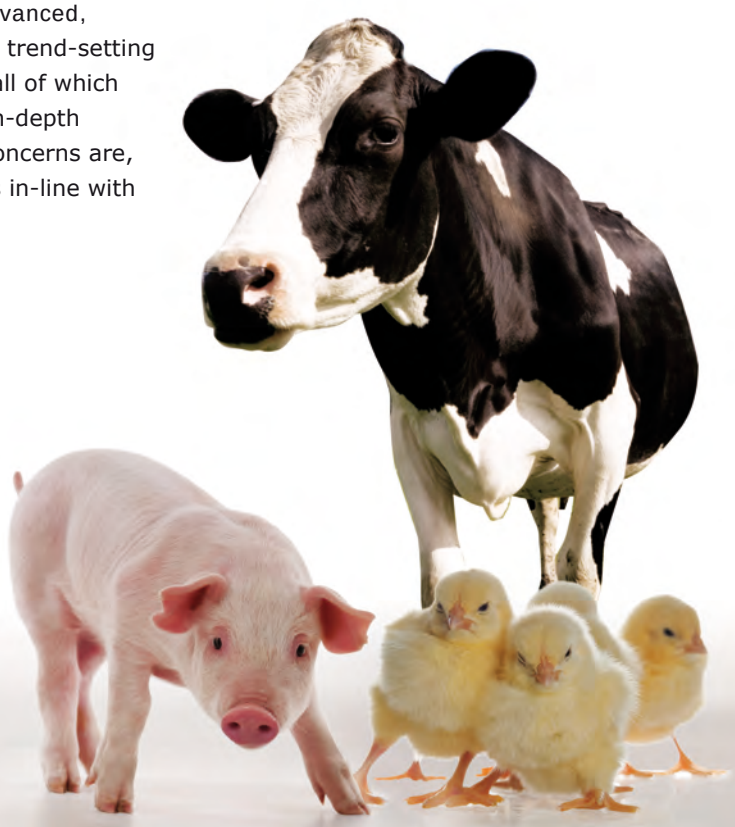

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BIOMIN Canada Inc
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Tel: 450-446-9434, Fax: 450-446-5118
e-mail: pierre.desmarais@biomin.net
e-mail: ron.gauthier@biomin.net

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Nutritional Requirement on the Immune Function of Livestock Species

Besoins nutritionnels de la fonction immunitaire chez les animaux d'élevage

S.K. Kvidera[1], E.A. Horst[1], M. Al-Qaisi[1], M.J. Dickson[1], R.P. Rhoads[2], A.F. Keating[1], and L.H. Baumgard[1]

[1]Iowa State University Department of Animal Science
[2]Virginia Tech Department of Animal Science
Corresponding author: baumgard@iastate.edu

Abstract

Metabolic maladaptation to lactation (ketosis) and heat stress are two of the most economically important pathologies in the dairy industry and both severely jeopardize the competitiveness of global animal agriculture. Heat stress and ketosis affect herds of all sizes and every dairy region in the country and world. The biology of ketosis and heat stress has been thoroughly studied for almost a half century, but the negative impacts of both (from an animal welfare and fiscal perspective) are as severe today as they were 30 years ago. We suggest, based upon the literature and our supporting evidence, that endotoxin is the common culprit etiological origin of both metabolic disorders.

Résumé

Le désordre d'adaptation à la lactation (acétonémie) et le stress thermique comptent parmi les pathologies qui présentent le plus d'importance économique pour l'industrie laitière et nuisent sérieusement à la compétitivité des productions animales en général. Le stress thermique et l'acétonémie touchent les troupeaux de toute taille dans toutes les régions laitières du pays et du monde. La biologie de l'acétonémie et du stress thermique a été rigoureusement étudiée depuis près d'un demi-siècle, mais leurs effets négatifs (aux points de vue du bien-être animal et fiscal) sont aussi graves aujourd'hui qu'il y a 30 ans. Nous croyons, d'après la littérature et nos propres observations, que l'endotoxine est l'agent étiologique commun aux deux désordres métaboliques.

Introduction

There are a variety of situations in an animal's life when nutrient utilization is reprioritized from productive towards agriculturally unproductive purposes. Two well-known examples that markedly reduce production are heat stress and ketosis. Decreased feed intake, experienced during both diseases, is unable to fully explain decreases in productivity. Additionally, both diseases are characterized by negative energy balance, body weight loss, inflammation, and

hepatic steatosis. While the metabolism of ketosis and heat stress have been thoroughly studied for the last 40 years, the initial insult in the cascade of events ultimately reducing productivity in both heat-stressed and ketotic cows has not been identified. To that end, we have generated preliminary data strongly implicating a metabolic disruptor, endotoxin, as the etiological culprit in each case.

Heat Stress

Heat stress negatively impacts a variety of production parameters and is a significant financial burden (~$900 million/year for dairy in the U.S. alone; St. Pierre et al., 2003). Heat-stress affects productivity indirectly by reducing feed intake; however, direct mechanisms also contribute as we have shown reduced feed intake only explains approximately 35-50% of the decreased milk yield during heat stress (Rhoads et al., 2009; Wheelock et al., 2010; Baumgard et al., 2011). Direct mechanisms contributing to heat stress milk yield losses involve an altered endocrine profile, including reciprocal changes in circulating anabolic and catabolic hormones (Bernabucci et al., 2010; Baumgard and Rhoads, 2012). Such changes are characterized by increased circulating insulin concentration, lack of adipose tissue lipid mobilization, and reduced adipocyte responsiveness to lipolytic stimuli. Hepatic and skeletal muscle cellular bioenergetics also exhibit clear differences in carbohydrate production and use, respectively, due to heat stress. Thus, the heat stress response markedly alters post-absorptive carbohydrate, lipid, and protein metabolism through coordinated changes in fuel supply and utilization across tissues in a manner distinct from commonly recognizable changes that occur in animals on a reduced plane of nutrition (Baumgard and Rhoads, 2013). The result of HS is underachievement of an animal's full genetic potential.

Ketosis

The periparturient period is associated with substantial metabolic changes involving normal homeorhetic adaptations to support milk production. Unfortunately, a disproportionate amount of herd culling occurs before cows reach 60 days in milk (Godden, 2003). Ketosis is defined as an excess of circulating ketone bodies and is characterized by decreases in feed intake, milk production, and increased risk of developing other transition period diseases (Chapinal et al., 2012). Epidemiological data indicate about 20% of transitioning dairy cows clinically experience ketosis (BHBA > 3.0 mM; Gillund et al., 2001) while the incidence of subclinical ketosis (>1.2 mM BHBA) is thought to be much higher (> 40%; McArt et al., 2012). Ketosis is a costly disorder (estimated at ~$300 per case; McArt et al., 2015) and thus it represents a major hurdle to farm profitability. Traditionally, ketosis is thought to result from excessive adipose tissue mobilization (Baird, 1982; Grummer, 1993; Drackley, 1999) which in turn contributes to fatty liver (hepatic steatosis) and excessive ketone body synthesis (Grummer, 1993).

Heat Stress Etiology

Mechanisms responsible for altered nutrient partitioning during HS are not clear; however, they might be mediated by HS effects on gastrointestinal health and function as we and others have demonstrated HS compromised intestinal barrier function (Lambert et al., 2002; Dokladny et al., 2006; Pearce et al., 2013; Sanz-Fernandez et al., 2014). During HS, blood flow is diverted from the viscera to the periphery in an attempt to dissipate heat leading to intestinal hypoxia (Hall et al., 1999). Enterocytes are particularly sensitive to hypoxia and nutrient restriction (Rollwagen et al.,

2006), resulting in ATP depletion and increased oxidative and nitrosative stress (Hall et al., 2001). This contributes to tight junction dysfunction and gross morphological changes that ultimately reduce intestinal barrier function (Lambert et al., 2002; Pearce et al., 2013). As a result, HS increases the passage of luminal content into portal and systemic blood (Hall et al., 2001; Pearce et al., 2013). Endotoxin, otherwise referred to as lipopolysaccharide (LPS), is a glycolipid embedded in the outer membrane of Gram-negative bacteria, which are abundant and prolific in luminal content, and is a well-characterized potent immune stimulator in multiple species (Berczi et al., 1966; Giri et al., 1990; Tough et al., 1997). Activation of the immune system occurs when LPS binding protein (LBP) initially binds LPS and together with CD14 and TLR4 delivers LPS for removal and detoxification, thus LBP is frequently used as a biomarker for LPS infiltration (Ceciliani et al., 2012). For a detailed description of how livestock and other species detoxify LPS see our recent review (Mani et al., 2012). Endotoxin infiltration during HS into the bloodstream which was first observed by Graber et al. (1971), is common among heat stroke patients (Leon, 2007) and is thought to play a central role in heat stroke pathophysiology as survival increases when intestinal bacterial load is reduced or when plasma LPS is neutralized (Bynum et al., 1979; Gathiram et al., 1987). It is remarkable how animals suffering from heat stroke or severe endotoxemia share many physiological and metabolic similarities to HS, such as an increase in circulating insulin (Lim et al., 2007). Infusing LPS into the mammary gland increased (~2 fold) circulating insulin in lactating cows (Waldron et al., 2006). In addition, we intravenously infused LPS into growing calves and pigs and demonstrated >10 fold increase in circulating insulin (Rhoads et al., 2009; Stoakes et al., 2015a; Kvidera et al., 2016). Interestingly, increased insulin occurs prior to increased inflammation and the temporal pattern agrees with our previous *in vivo* data and a recent *in vitro* report (Bhat et al., 2014) suggesting LPS stimulates insulin secretion, either directly or via GLP-1 (Kahles et al., 2014). The possibility that LPS increases insulin secretion likely explains the hyperinsulinemia we have repeatedly reported in a variety of heat-stressed agriculture models (Baumgard and Rhoads, 2013). Again, the increase in insulin in both models is energetically difficult to explain as feed intake was severely depressed in both experiments.

Transition Period Inflammation

Recently, the concept that LPS impacts normal nutrient partitioning and potentially contributes to metabolic maladaptation to lactation has started to receive attention. Although LPS itself has not been the primary causative focus, general inflammation has been the topic of investigations. Increased inflammatory markers following parturition have been reported in cows (Ametaj et al., 2005; Bertoni et al., 2008; Humblet et al., 2006; Mullins et al., 2012). Presumably, the inflammatory state following calving disrupts normal nutrient partitioning and is detrimental to productivity (Loor et al., 2005; Bertoni et al., 2008), and this assumption was recently reinforced when TNFα infusion decreased productivity (albeit without overt changes in metabolism; Yuan et al., 2013; Martel et al., 2014). Additionally, in late-lactation cows, injecting TNFα increased (>100%) liver TAG content without a change in circulating NEFA (Bradford et al., 2009). Our recent data demonstrates increased inflammatory markers in cows diagnosed with ketosis only and no other health disorders. In comparison with healthy controls, ketotic cows had increased circulating LPS prior to calving and post-partum acute phase proteins such as LPS-binding protein, serum amyloid A, and haptoglobin were also increased (Fig. 1; Abuajamieh et al., 2015).

Endotoxin can originate from a variety of locations, and obvious sources in transitioning dairy cows include the uterus (metritis), mammary gland (mastitis) and the gastrointestinal tract (Mani et al., 2012). However, we believe intestinal permeability may be responsible for inflammation observed in the transition dairy cow. A transitioning dairy cow undergoes a post-calving diet shift from a mainly forage based to a high concentrate ration. This has the potential to induce rumen acidosis which can compromise the gastrointestinal tract barrier (Khafipour et al., 2009).

In order to further investigate the effects of intestinal permeability on production and inflammation, we intentionally induced intestinal permeability in mid-lactation dairy cows using a gamma secretase inhibitor (GSI), a compound that specifically inhibits crypt stem cell differentiation into enterocytes via disrupting Notch signaling (van Es et al., 2005). We anticipated feed intake of GSI administered cows would decrease, so we pair-fed controls in order to eliminate the confounding effect of feed intake. Treatment with GSI decreased feed intake and altered jejunum morphology consistently with characteristics of leaky gut (shortened crypt depth, decreased villus height, decreased villus height to crypt depth ratio). Circulating insulin and LBP were increased in GSI cows relative to controls. Interestingly in our GSI model, acute phase proteins serum amyloid A and haptoglobin increased for both treatments over time, indicating inflammation was occurring in pair-fed controls as well (Stoakes et al., 2014). This is not surprising, as pair-fed controls were receiving ~20% of their ad libitum intake and decreased feed intake has been shown to increase intestinal permeability in feed restricted rodents and humans (Rodriguez et al., 1996; Welsh et al., 1998) and we have also observed this in pigs (Pearce et al., 2013; Sanz-Fernandez et al., 2014). Recently, we confirmed the detrimental effects of feed restriction in mid-lactation cows by demonstrating a linear increase in circulating acute phase proteins and endotoxin with increasing severity of feed restriction. Furthermore, cows fed 40% of ad libitum intake had shortened ileum villus height and crypt depth, indicating reduced intestinal health (Stoakes et al., 2015b). In summary, inflammation is present during the transition period and likely contributes to changes in whole-animal energetics.

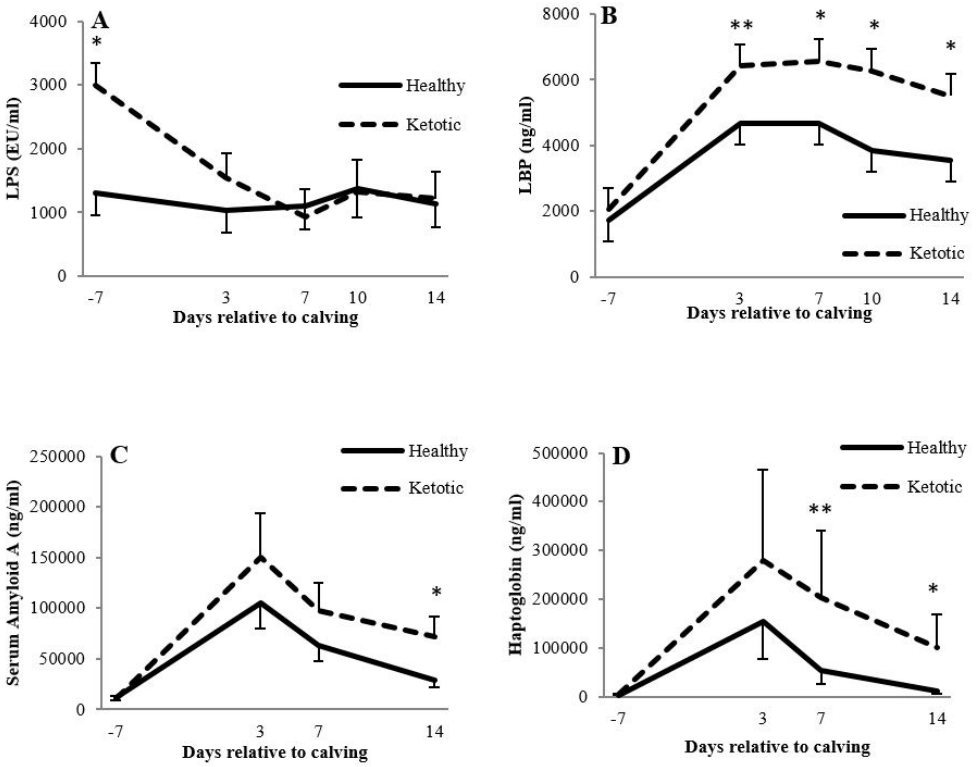


Figure 1. Markers of inflammation in healthy (solid line) and ketotic (dashed line) transition cows (Abuajamieh et al., 2016).

Metabolism of Inflammation

LPS-induced inflammation has an energetic cost which redirects nutrients away from anabolic process that support milk and muscle synthesis (see review by Johnson, 1997, 1998) and thus compromises productivity and efficiency. Interestingly, immune cells become more insulin sensitive and consume copious amounts of glucose upon activation in order to support rapid proliferation and biosynthetic processes (Calder et al., 2007; Palsson-McDermott and O'Neill, 2013). In contrast, inflammation induces an insulin resistant state in skeletal muscle and adipose tissue (Liang et al., 2013; Poggi et al., 2007). Recent data has also demonstrated a decrease in ketone oxidation during LPS infiltration (Suagee et al., 2011; Frisard et al., 2015) which we believe may partly explain increased ketone body concentrations during the transition period.

Endotoxin has previously been recognized to be involved with metabolic dysfunction. In humans, both obesity and high fat diets are linked to endotoxemia (Cani et al., 2007, Gregor and Hotamisligil, 2011). Furthermore, LPS is involved with the development of fatty liver (Ilan, 2012), and cytokines are linked to lipid accumulation and cholesterol retention (Ma et al., 2008; Clément et al., 2008). Experimentally-induced endotoxemia in dairy cattle has been linked to several metabolic and endocrine disturbances including decreased circulating glucose, termination of pregnancy, leukopenia, disruption of ruminal metabolism, and altered calcium homeostasis (Griel et al., 1975; Giri et al., 1990; Waldron et al., 2003; Jing et al., 2014). The aforementioned pathological conditions are likely mediated by LPS-induced inflammation and the subsequent changes in nutrient partitioning caused by immune system activation.

Energetic Cost of Immune Activation

An activated immune system requires a large amount of energy and the literature suggests that glucose homeostasis is markedly disrupted (Leininger et al., 2000) during an endotoxin challenge. Upon immune system activation, immune cells switch their metabolism from oxidative phosphorylation to aerobic glycolysis, causing them to become obligate glucose utilizers in a phenomenon known as the Warburg Effect (Vander Hiden et al., 2009). Our group recently employed a series of LPS-euglycemic clamps to quantify the energetic cost of an activated immune system. Using this model, we estimated approximately 1 kg of glucose is used by the immune system during a 12 hour period in lactating dairy cows. Interestingly, on a metabolic body weight basis the amount of glucose utilized by LPS-activated immune system in lactating cows, growing steers and growing pigs were 0.66, 1.0, and 1.1 g glucose/kg $BW^{0.75}$/h, respectively; Stoakes et al., 2015; Kvidera et al., 2016,2017). Increased immune system glucose utilization occurs simultaneously with infection-induced decreased feed intake: this coupling of enhanced nutrient requirements with hypophagia obviously decrease the amount of nutrients available for the synthesis of valuable products (milk, meat, fetus, wool). We and others have now demonstrated that both heat-stressed and ketotic animals have increased circulating markers of endotoxin and inflammation. We believe that the circulating LPS in both maladies originates from the intestine and thus both likely have an activated immune system. This activated systemic immune response reprioritizes the hierarchy of glucose utilization and milk synthesis is consequently deemphasized.

Conclusion

Ketosis and heat stress are two of the most economically important pathologies which severely jeopardize the competitiveness of animal agriculture. Heat stress and ketosis affect herds of all sizes and every dairy region in country. The biology of ketosis and heat stress has been studied for almost a half century, but the negative impacts of both are as severe today as they were 30 years ago. We suggest, based upon the literature and on our supporting evidence, that LPS is the common culprit etiological origin of both metabolic disorders. Taken together, our data and the literature suggest that LPS markedly alters nutrient partitioning and is a causative agent in metabolic disruption during heat stress and ketosis.

References

Abuajamieh, M., S. K. Kvidera, M. V. Fernandez, A. Nayeri, N. C. Upah, E. A. Nolan, S. M. Lei, J. M. DeFrain, H. B. Green, K. M. Schoenberg, W. E. Trout, and L. H. Baumgard. 2016. Inflammatory biomarkers are associated with ketosis in periparturient Holstein cows. Res. Vet. Sci. 109:81-85.

Ametaj, B. N., B. J. Bradford, G. Bobe, R. A. Nafikov, Y. Lu, J. W. Young, and D. C. Beitz. 2005. Strong relationships between mediators of the acute phase response and fatty liver in dairy cows. Can. J. Anim. Sci. 85:165-175.

Baird, G. D. 1982. Primary ketosis in the high-producing dairy cow: clinical and subclinical disorders, treatment, prevention, and outlook. J. Dairy Sci. 65:1-10.

Baumgard, L. and R. Rhoads. 2013. Effects of heat stress on postabsorptive metabolism and energetics. Annu. Rev. Anim. Biosci. 1:7.1-7.27.

Baumgard, L. H. and R. P. Rhoads. 2012. Ruminant Nutrition Symposium: ruminant production and metabolic responses to heat stress. J. Anim. Sci. 90:1855-1865.

Baumgard, L. H., J. B. Wheelock, S. R. Sanders, C. E. Moore, H. B. Green, M. R. Waldron, and R. P. Rhoads. 2011. Postabsorptive carbohydrate adaptations to heat stress and monensin supplementation in lactating Holstein cows. J. Dairy Sci. 94:5620-5633.

Berczi, I., L. Bertok, and T. Bereznai. 1966. Comparative studies on the toxicity of Escherichia coli lipopolysaccharide endotoxin in various animal species. Can. J. Microbiol. 12:1070-1071.

Bernabucci, U., N. Lacetera, L. H. Baumgard, R. P. Rhoads, B. Ronchi, and A. Nardone. 2010. Metabolic and hormonal acclimation to heat stress in domesticated ruminants. Animal 4:1167-1183.

Bertoni, G., E. Trevisi, X. Han, and M. Bionaz. 2008. Effects of inflammatory conditions on liver activity in puerperium period and consequences for performance in dairy cows. J. Dairy Sci. 91:3300-3310.

Bhat, U. G., V. Ilievski, T. G. Unterman, and K. Watanabe. 2014. Porphyromonas gingivalis lipopolysaccharide upregulates insulin secretion from pancreatic β cell line MIN6. J. Periodontol. 85:1629-1636.

Bradford, B. J., L. K. Mamedova, J. E. Minton, J. S. Drouillard, and B. J. Johnson. 2009. Daily injection of tumor necrosis factor-{alpha} increases hepatic triglycerides and alters transcript abundance of metabolic genes in lactating dairy cattle. J. Nutr. 139:1451-1456.

Bynum, G., J. Brown, D. Dubose, M. Marsili, I. Leav, T. G. Pistole, M. Hamlet, M. LeMaire, and B. Caleb. 1979. Increased survival in experimental dog heatstroke after reduction of gut flora. Aviat. Space Environ. Med. 50:816-819.

Calder, P. C., G. Dimitriadis, and P. Newsholme. 2007. Glucose metabolism in lymphoid and inflammatory cells and tissues. Curr. Opin. Clin. Nutr. Metab. Care 10:531-540.

Cani, P. D., J. Amar, M. A. Iglesias, M. Poggi, C. Knauf, D. Bastelica, A. M. Neyrinck, F. Fava, K. M. Tuohy, C. Chabo, A. Waget, E. Delmée, B. Cousin, T. Sulpice, B. Chamontin, J. Ferrières, J. F. Tanti, G. R. Gibson, L. Casteilla, N. M. Delzenne, M. C. Alessi, and R. Burcelin. 2007. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes 56:1761-1772.

Ceciliani, F., J. J. Ceron, P. D. Eckersall, and H. Sauerwein. 2012. Acute phase proteins in ruminants. J. Proteomics 75:4207-4231.

Chapinal, N., S. J. Leblanc, M. E. Carson, K. E. Leslie, S. Godden, M. Capel, J. E. Santos, M. W. Overton, and T. F. Duffield. 2012. Herd-level association of serum metabolites in the transition period with disease, milk production, and early lactation reproductive performance. J. Dairy Sci. 95:5676-5682.

Clément, S., C. Juge-Aubry, A. Sgroi, S. Conzelmann, V. Pazienza, B. Pittet-Cuenod, C. A. Meier, and F. Negro. 2008. Monocyte chemoattractant protein-1 secreted by adipose tissue induces direct lipid accumulation in hepatocytes. Hepatology 48:799-807.

Dokladny, K., P. L. Moseley, and T. Y. Ma. 2006. Physiologically relevant increase in temperature causes an increase in intestinal epithelial tight junction permeability. Am. J. Physiol. Gastrointest. Liver Physiol. 290:G204-212.

Drackley, J. K. 1999. ADSA Foundation Scholar Award. Biology of dairy cows during the transition period: the final frontier? J. Dairy Sci. 82:2259-2273.

Frisard, M. I., R. P. McMillan, J. Marchand, K. A. Wahlberg, Y. Wu, K. A. Voelker, L. Heilbronn, K. Haynie, B. Muoio, L. Li, and M. W. Hulver. 2010. Toll-like receptor 4 modulates skeletal muscle substrate metabolism. Am. J. Physiol. Endocrinol. Metab. 298:E988-998.

Gathiram, P., M. T. Wells, J. G. Brock-Utne, and S. L. Gaffin. 1987. Antilipopolysaccharide improves survival in primates subjected to heat stroke. Circ. Shock 23:157-164.

Gillund, P., O. Reksen, Y. T. Gröhn, and K. Karlberg. 2001. Body condition related to ketosis and reproductive performance in Norwegian dairy cows. J. Dairy Sci. 84:1390-1396.

Giri, S. N., P. Emau, J. S. Cullor, G. H. Stabenfeldt, M. L. Bruss, R. H. Bondurant, and B. I. Osburn. 1990. Effects of endotoxin infusion on circulating levels of eicosanoids, progesterone, cortisol, glucose and lactic acid, and abortion in pregnant cows. Vet. Microbiol. 21:211-231.

Godden, S. M., S. C. Stewart, J. F. Fetrow, P. Rapnicki, R. Cady, W. Weiland, H. Spencer, and S. W. Eicker. 2003. The relationship between herd rbSTsupplementation and other factors and risk for removal for cows in Minnesota Holstein dairy herds. Pp. 55-64 in Proc. Four-State Nutrition Conference. MidWest Plan Service, LaCrosse, WI.

Graber, C. D., R. B. Reinhold, J. G. Breman, R. A. Harley, and G. R. Hennigar. 1971. Fatal heat stroke. Circulating endotoxin and gram-negative sepsis as complications. JAMA 216:1195-1196.

Gregor, M. F. and G. S. Hotamisligil. 2011. Inflammatory mechanisms in obesity. Annu. Rev. Immunol. 29:415-445.

Griel, L. C., A. Zarkower, and R. J. Eberhart. 1975. Clinical and clinico-pathological effects of Escherichia coli endotoxin in mature cattle. Can. J. Comp. Med. 39:1-6.

Grummer, R. R. 1993. Etiology of lipid-related metabolic disorders in periparturient dairy cows. J. Dairy Sci. 76:3882-3896.

Hall, D. M., K. R. Baumgardner, T. D. Oberley, and C. V. Gisolfi. 1999. Splanchnic tissues undergo hypoxic stress during whole body hyperthermia. Am. J. Physiol. 276:G1195-1203.

Hall, D. M., G. R. Buettner, L. W. Oberley, L. Xu, R. D. Matthes, and C. V. Gisolfi. 2001. Mechanisms of circulatory and intestinal barrier dysfunction during whole body hyperthermia. Am. J. Physiol. Heart Circ. Physiol. 280:H509-521.

Humblet, M. F., H. Guyot, B. Boudry, F. Mbayahi, C. Hanzen, F. Rollin, and J. M. Godeau. 2006. Relationship between haptoglobin, serum amyloid A, and clinical status in a survey of dairy herds during a 6-month period. Vet. Clin. Pathol. 35:188-193.

Ilan, Y. 2012. Leaky gut and the liver: a role for bacterial translocation in nonalcoholic steatohepatitis. World J. Gastroenterol. 18:2609-2618.

Jing, L., R. Zhang, Y. Liu, W. Zhu, and S. Mao. 2014. Intravenous lipopolysaccharide challenge alters ruminal bacterial microbiota and disrupts ruminal metabolism in dairy cattle. Br. J. Nutr. 112:170-182.

Johnson, R. W. 1997. Inhibition of growth by pro-inflammatory cytokines: an integrated view. J. Anim. Sci. 75:1244-1255.

Johnson, R. W. 1998. Immune and endocrine regulation of food intake in sick animals. Domest. Anim. Endocrinol. 15:309-319.

Kahles, F., C. Meyer, J. Möllmann, S. Diebold, H. M. Findeisen, C. Lebherz, C. Trautwein, A. Koch, F. Tacke, N. Marx, and M. Lehrke. 2014. GLP-1 secretion is increased by inflammatory stimuli in an IL-6-dependent manner, leading to hyperinsulinemia and blood glucose lowering. Diabetes 63:3221-3229.

Khafipour, E., D. O. Krause, and J. C. Plaizier. 2009. A grain-based subacute ruminal acidosis challenge causes translocation of lipopolysaccharide and triggers inflammation. J. Dairy Sci. 92:1060-1070.

Kvidera, S. K., E. A. Horst, M. Abuajamieh, E. J. Mayorga, M. V. Sanz Fernandez, and L. H. Baumgard. 2016. Technical note: A procedure to estimate glucose requirements of an activated immune system in steers. J. Anim. Sci. 94:4591-4599.

Kvidera, S. K., E. A. Horst, M. Abuajamieh, E. J. Mayorga, M. V. Sanz Fernandez, and L. H. Baumgard. 2017. Glucose requirements of an activated immune system in lactating Holstein cows. J. Dairy Sci. 100:2360-2374.

Lambert, G. P., C. V. Gisolfi, D. J. Berg, P. L. Moseley, L. W. Oberley, and K. C. Kregel. 2002. Selected contribution: Hyperthermia-induced intestinal permeability and the role of oxidative and nitrosative stress. J. Appl. Physiol. 92:1750-1761.

Leininger, M. T., C. P. Portocarrero, A. P. Schinckel, M. E. Spurlock, C. A. Bidwell, J. N. Nielsen, and K. L. Houseknecht. 2000. Physiological response to acute endotoxemia in swine: effect of genotype on energy metabolites and leptin. Domest. Anim. Endocrinol. 18:71-82.

Leon, L. R. 2007. Heat stroke and cytokines. Prog. Brain Res. 162:481-524.

Liang, H., S. E. Hussey, A. Sanchez-Avila, P. Tantiwong, and N. Musi. 2013. Effect of lipopolysaccharide on inflammation and insulin action in human muscle. PLoS One 8:e63983.

Lim, C. L., G. Wilson, L. Brown, J. S. Coombes, and L. T. Mackinnon. 2007. Pre-existing inflammatory state compromises heat tolerance in rats exposed to heat stress. Am. J. Physiol. Regul. Integr. Comp. Physiol. 292:R186-194.

Loor, J. J., H. M. Dann, R. E. Everts, R. Oliveira, C. A. Green, N. A. Guretzky, S. L. Rodriguez-Zas, H. A. Lewin, and J. K. Drackley. 2005. Temporal gene expression profiling of liver from periparturient dairy cows reveals complex adaptive mechanisms in hepatic function. Physiol. Genomics 23:217-226.

Ma, K. L., X. Z. Ruan, S. H. Powis, Y. Chen, J. F. Moorhead, and Z. Varghese. 2008. Inflammatory stress exacerbates lipid accumulation in hepatic cells and fatty livers of apolipoprotein E knockout mice. Hepatology 48:770-781.

Mani, V., T. E. Weber, L. H. Baumgard, and N. K. Gabler. 2012. Growth and Development Symposium: Endotoxin, inflammation, and intestinal function in livestock. J. Anim. Sci. 90:1452-1465.

Martel, C. A., L. K. Mamedova, J. E. Minton, M. L. Jones, J. A. Carroll, and B. J. Bradford. 2014. Continuous low-dose infusion of tumor necrosis factor alpha in adipose tissue elevates adipose tissue interleukin 10 abundance and fails to alter metabolism in lactating dairy cows. J. Dairy Sci. 97:4897-4906.

McArt, J. A., D. V. Nydam, and G. R. Oetzel. 2012. Epidemiology of subclinical ketosis in early lactation dairy cattle. J. Dairy Sci. 95:5056-5066.

McArt, J. A., D. V. Nydam, and M. W. Overton. 2015. Hyperketonemia in early lactation dairy cattle: A deterministic estimate of component and total cost per case. J. Dairy Sci. 98:2043-2054.

Mullins, C. R., L. K. Mamedova, M. J. Brouk, C. E. Moore, H. B. Green, K. L. Perfield, J. F. Smith, J. P. Harner, and B. J. Bradford. 2012. Effects of monensin on metabolic parameters, feeding behavior, and productivity of transition dairy cows. J. Dairy Sci. 95:1323-1336.

Palsson-McDermott, E. M. and L. A. O'Neill. 2013. The Warburg effect then and now: from cancer to inflammatory diseases. Bioessays 35:965-973.

Pearce, S. C., V. Mani, T. E. Weber, R. P. Rhoads, J. F. Patience, L. H. Baumgard, and N. K. Gabler. 2013. Heat stress and reduced plane of nutrition decreases intestinal integrity and function in pigs. J. Anim. Sci. 91:5183-5193.

Poggi, M., D. Bastelica, P. Gual, M. A. Iglesias, T. Gremeaux, C. Knauf, F. Peiretti, M. Verdier, I. Juhan-Vague, J. F. Tanti, R. Burcelin, and M. C. Alessi. 2007. C3H/HeJ mice carrying a toll-like receptor 4 mutation are protected against the development of insulin resistance in white adipose tissue in response to a high-fat diet. Diabetologia 50:1267-1276.

Rhoads, M., R. Rhoads, M. VanBaale, R. Collier, S. Sanders, W. Weber, B. Crooker, and L. Baumgard. 2009. Effects of heat stress and plane of nutrition on lactating Holstein cows: I. Production, metabolism, and aspects of circulating somatotropin. J. Dairy Sci. 92:1986-1997.

Rodriguez, P., N. Darmon, P. Chappuis, C. Candalh, M. A. Blaton, C. Bouchaud, and M. Heyman. 1996. Intestinal paracellular permeability during malnutrition in guinea pigs: effect of high dietary zinc. Gut 39:416-422.

Rollwagen, F. M., S. Madhavan, A. Singh, Y. Y. Li, K. Wolcott, and R. Maheshwari. 2006. IL-6 protects enterocytes from hypoxia-induced apoptosis by induction of bcl-2 mRNA and reduction of fas mRNA. Biochem. Biophys. Res. Commun. 347:1094-1098.

Sanz Fernandez, M. V., S. C. Pearce, N. K. Gabler, J. F. Patience, M. E. Wilson, M. T. Socha, J. L. Torrison, R. P. Rhoads, and L. H. Baumgard. 2014. Effects of supplemental zinc amino acid complex on gut integrity in heat-stressed growing pigs. Animal 8:43-50.

St. Pierre, N., B. Cobanov, and G. Schnitkey. 2003. Economic losses from heat stress by US livestock industries. J. Dairy Sci. 86:E52-E77.

Stoakes, S. K., E. A. Nolan, M. Abuajamieh, M. V. Sanz Fernandez, and L. H. Baumgard. 2015a. Estimating glucose requirements of an activated immune system in growing pigs. J. Anim. Sci. 93:634.

Stoakes, S. K., E. A. Nolan, D .J. Valko, M. Abuajamieh, J. T. Seibert, M. V. Sanz Fernandez, P. J. Gorden, H. B. Green, K. M. Schoenberg, W. E. Trout, and L. H. Baumgard. 2015b. Characterizing the effect of feed restriction on biomarkers of leaky gut. J. Dairy Sci. 98:274. (Abstr.).

Suagee, J. K., B. A. Corl, J. G. Wearn, M. V. Crisman, M. W. Hulver, R. J. Geor, and L. J. McCutcheon. 2011. Effects of the insulin-sensitizing drug pioglitazone and lipopolysaccharide administration on insulin sensitivity in horses. J. Vet. Intern. Med. 25:356-364.

Tough, D. F., S. Sun, and J. Sprent. 1997. T cell stimulation in vivo by lipopolysaccharide (LPS). J. Exp. Med. 185:2089-2094.

van Es, J. H., M. E. van Gijn, O. Riccio, M. van den Born, M. Vooijs, H. Begthel, M. Cozijnsen, S. Robine, D. J. Winton, F. Radtke, and H. Clevers. 2005. Notch/gamma-secretase inhibition turns proliferative cells in intestinal crypts and adenomas into goblet cells. Nature 435:959-963.

Vander Heiden, M. G., L. C. Cantley, and C. B. Thompson. 2009. Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science 324:1029-1033.

Waldron, M. R., A. E. Kulick, A. W. Bell, and T. R. Overton. 2006. Acute experimental mastitis is not causal toward the development of energy-related metabolic disorders in early postpartum dairy cows. J. Dairy Sci. 89:596-610.

Waldron, M. R., B. J. Nonnecke, T. Nishida, R. L. Horst, and T. R. Overton. 2003. Effect of lipopolysaccharide infusion on serum macromineral and vitamin D concentrations in dairy cows. J. Dairy Sci. 86:3440-3446.

Welsh, F. K., S. M. Farmery, K. MacLennan, M. B. Sheridan, G. R. Barclay, P. J. Guillou, and J. V. Reynolds. 1998. Gut barrier function in malnourished patients. Gut 42:396-401.

Wheelock, J., R. Rhoads, M. Vanbaale, S. Sanders, and L. Baumgard. 2010. Effects of heat stress on energetic metabolism in lactating Holstein cows. J. Dairy Sci. 93:644-655.

Yuan, K., J. K. Farney, L. K. Mamedova, L. M. Sordillo, and B. J. Bradford. 2013. TNFα altered inflammatory responses, impaired health and productivity, but did not affect glucose or lipid metabolism in early-lactation dairy cows. PLoS One 8:e80316.

Nutritional and Management Challenges and Solutions in ABF Swine Production

Solutions aux problèmes de nutrition et de gestion dans les élevages porcins exempts d'antibiotiques

Laura L. Greiner
Nutrition Director, Carthage Veterinary Service, Ltd, Carthage, IL 62321.
lgreiner@hogvet.com

Abstract

Antibiotic free programs have been around for many years. Some programs are very successful. The success or failure of the program is dependent upon preventing pathogen entry and providing a nutrition program that focuses on boosting gut and systemic immunity. While many farmers do not use antibiotics in finishing and sow rations, starting newly weaned pigs with developing immune systems and changing intestinal tracts can be a challenge. Much research has been conducted looking at different methods for starting pigs to prevent enteric challenges and overcoming systemic pathogens. Recently, work is also focusing on ingredients that can be added to both sow and finishing diets to improve gut health and minimize pathogen transmission. The goal of today's discussion is to focus on processes and nutritional programs to maintain good animal growth/reproduction performance and animal health while using antibiotic free programs.

Résumé

L'élevage sans antibiotiques se pratique depuis de nombreuses années, parfois avec beaucoup de succès d'ailleurs. La réussite ou l'échec d'un programme d'élevage sans antibiotiques repose sur l'aptitude à prévenir l'entrée des agents pathogènes et à fournir un programme nutritionnel axé sur la stimulation de l'immunité gastro-intestinale et systémique. Bien que de nombreux éleveurs n'utilisent aucun antibiotique dans les rations de finition ni dans celles des truies, le démarrage des porcelets sevrés peut s'avérer difficile dans le contexte où leur système immunitaire est en formation et leur tube digestif subit des changements. Beaucoup de recherche a été effectuée pour examiner différentes méthodes de démarrage des porcelets permettant de prévenir les troubles entériques et de combattre les agents pathogènes systémiques. Depuis peu, on étudie aussi certains ingrédients qui pourraient être ajoutés aux rations des truies et des porcs en finition pour améliorer la santé intestinale et réduire au maximum la transmission des agents pathogènes. L'objectif de la présente communication est d'examiner les processus et programmes nutritionnels qui permettront de conserver les bonnes performances zootechniques de croissance/ reproduction et la santé des animaux élevés sans antibiotiques.

Introduction

The use of antibiotics in the livestock industry has been in practice for many years. The evolution of antibiotic free (ABF) production has been directed both by the consumer and the health industry. Over the last 20 years, discussions on antibiotic resistant organisms in human medicine have evolved into the livestock industry to help control potential bacterial resistance over time across all species. In addition, recent consumer groups have also started to request that meat animals not be fed antibiotics during the course of their growth which is motivated by personal health, taste, quality and environmental concerns. While we acknowledge that for animal welfare, antibiotic intervention does need to be available to help control an illness, many producers are working towards either further reducing their use of antibiotics or raising a majority of their animals as ABF to meet the demands of the public.

Defining "Antibiotic Free"

The definition of antibiotic free (ABF) has many different versions. While animals cannot go to harvest if they have not had proper antibiotic withdrawals to minimize antibiotic residue detection potential, two other classifications of ABF currently exist. The first classification would be that of no antibiotics will be allowed after the pig is weaned from its mother. The second classification is defined as the "never-ever" category. Never-ever refers to the fact that the pig is never given an antibiotic during the course of its lifetime. In the United States today, both classifications of ABF are used for different markets.

In Canada, the Canadian Food Inspection Agency (CFIA) website states: "In order to display the claim raised without the use of antibiotics, the animal or fish must not have received antibiotics from birth to harvest. In addition, no antibiotics can be administered to the mother of the animal in question in any manner which would result in antibiotic residue in the animal. Vitamins and minerals given to the animal may only be given at the level of physiological action for dietary supplement, not for antimicrobial effect."

Defining the cost of antibiotic free

While there have been a few studies conducted that show the potential impact of ABF programs in production, the results are quite variable. According to Vansickle (2011), Dr. Main demonstrated that antibiotic free production in the US could cost on average $11/pig and go as high as $15.50 considering 70% of the barn will go to finishing as antibiotic free. Given that herd health and stability are major variables in swine production, the cost of ABF production would likely be highly variable even within a system. A 70% program completion rate could be easily achieved in some barns, but a major challenge in others. In 2008, an article was published at the American Association of Swine Veterinarians conference demonstrating that a facility can achieve equal reproductive performance and have minimal reductions in wean to finish performance without the use of antibiotics and animal proteins (Kohler et al., 2008). With the opportunity to capture an ABF premium, the producers were able to only have an increase in production costs of $0.32/head compared to the estimated European value of $5.24 at the time. While the data is not conclusive, what is conclusive is that good initial health and vaccine programs are keys to making the programs effective.

Defining problems and solutions in achieving antibiotic free

In the past, antibiotics have been used generally for one of three purposes: 1. Treat/manage disease, 2. Prevent disease, 3. Improve feed efficiency.

Whether a system chooses to have an ABF program or continues to use antibiotics to control disease, the focus of all swine farmers is to minimize the use of antibiotics and to prevent disease. There are four key areas associated with the practice of good animal health. These areas include: health programs, management, genetics, and nutrition.

Animal health programs and management practices go hand-in-hand. Animal caretakers must implement and effectively follow health programs outlined by the herd veterinarian. Herd veterinarians will establish vaccine strategies to minimize disease outbreaks and develop sanitation programs to reduce the spread of disease (biosecurity). Animal caretakers must carefully follow these programs as well as establish good biosecurity programs and sound rodent control. In addition, animal caretakers need to evaluate the animal facilities and maintain those facilities as part of routine maintenance and upkeep. Ventilation needs to be established by phase to reduce drafts on small pigs and also minimize any manure gases that may reduce performance. Selection of robust genetics that can tolerate a variety of environmental parameters will also improve the success of an ABF program.

Nutrition programs can have a significant impact on pig performance. In addition, ingredients other than antibiotics can be added to diets to help control or mitigate potential health challenges as the pig grows. In order to be effective in developing a nutritional program, nutritionists need to communicate with the farmer or the veterinarian to further understand the health status of the pig and also identify times during the piglet's growth phase where health challenges may arise. While there are many ingredients on the market that claim to aid in gut health, digestion, feed efficiency, and feed intake, understanding the mode of action and expected outcome will aid in the nutritionists decision process.

At weaning, the gut of the piglet goes through dramatic changes as the pig transitions from liquid feed to dry feed. Even in systems that maintain liquid feeding, the physiology of the gut changes in response to the weaning process. The focus the first 3 weeks after weaning is to transition pigs rapidly to their new food source and maintain a healthy gut and immune system to help the pig counter any pathogens that may be present as the maternal antibody declines.

Gut health for the weaned pig is an area that has been researched for many years. However, the dynamics of gut immunity, gut enzymes, and the microflora interactions are not well understood. Establishing a good population of beneficial gut bacteria that aid in digestion and reduce pathogenic bacteria balances around pH and substrates. Enzyme production is mainly that of lactase while other carbohydrase enzymes will increase through week 8 of age. Diets that are high in non-digestible feedstuffs allow significant nutrients to gut microflora, which increases for the potential of pathogens to overtake population and cause disease. Feeding highly digestible starches in early rations have been shown to improve gut health. In addition, in a series of studies conducted by Pettigrew and his research team, various plant extracts have also been

shown to be beneficial in improving intestinal villi length and reducing pathogenic bacteria. The use of probiotics to maintain a certain population of microorganisms has been shown to be beneficial. In addition, the use of lactose to lower the pH and maintain *Lactobacilli* has also been shown to improve pig performance.

As the piglet reaches 6 weeks of age, maternal antibodies start to decay and the pig reaches a susceptible period for health challenges. Song et al (2012) demonstrated that using egg antibodies can improve general health in populations of pigs with unspecified health status. While growth performance has been shown in some studies to be similar to spray-dried plasma (SDP), SDP has additional benefits in improving immune function (macrophage modulation) and cell junctions (Campbell et al., 2010).

Many farmers focus on minimizing late finishing pig mortality and feed efficiency. A recent study reported that enzymes (such as xylanase) have the potential to improve pig viability from 3.99% to 2.39% and improve gain:feed from 0.286 to 0.290 by converting arabinoxylans to xylo-oligomers which can shift the microflora to favor the beneficial organisms (Zier-Rush, 2016). In addition, Greiner et al. (2016) demonstrated that feeding 150 ppm of supplemental copper in late finishing improved gain:feed from 0.31 to 0.34. Sometimes, altering the feedstuffs can improve the health of the pig in the face of a health challenge. For many years, the human health groups have discussed the isoflavones and other beneficial components of soybeans. Data from Greiner et al (1999) and Rochell et al (2015) demonstrated that feeding either isoflavones directly or higher levels of soybean meal to pigs in the face of a PRRS infection can reduce viral replication and improve performance.

While much research has demonstrated that antibiotics can improve feed efficiency, there are also mechanical, genetic, environmental, and nutritional modifications that can also improve feed efficiency. In Table 1, as particle size reduces from 650µm to 350µm, finishing average daily feed intake decreased and gain:feed increased. Pelleting feed resulted in improved average daily gain. However, pelleting 350µm corn versus 650µm corn showed no additional benefit (Nemechek et al., 2016). Other studies have demonstrated on average a 4-8% improvement in both average daily gain and feed efficiency when diets are pelleted in grow finish (Miller, 2012).

Table 1. The impact of particle size and feed form on finishing swine production parameters. Nemecheck et al., 2016.

	Meal			Pellet		
	650µ	50% 650µ + 50% 350µ	350µ	650µ	50% 650µ + 50% 350µ	350µ
ADG, kg	0.90	0.89	0.89	0.94	0.93	0.92
ADFI, kg	2.41	2.34	2.26	2.35	2.37	2.35
G:F	0.372	0.375	0.382	0.399	0.392	0.391

In addition, other factors can influence feed efficiency. Animals housed in an environment above or below their thermal neutral zone can have poorer rates of feed conversion. The health status of the animal can also alter feed efficiency and growth rate, as well as, genetics, and microbial loading of the environment.

References

Campbell, J.M., J. Polo, L.E. Russell, and J.D. Crenshaw. 2010. Review of spray-dried plasma's impact on intestinal barrier function. Livestock Science 133:239-241.

Greiner, L., A. Graham, B. Knopf, R.J. Harrell, and J. Connor. 2016. Evaluation of copper source addition in the grow-finish phase. Midwest American Society of Animal Science Meetings, Des Moines. Abstract #154.

Greiner, L., T. Stahly, and T.J. Stabel. 2001. The effect of dietary soy genistein on pig growth and viral replication during a viral challenge. Journal of Animal Science 79(5):1272-1279.

Kohler, D., J. Schneider, and C. Bierman. 2008. Profitable antibiotic-free pork production. North Carolina Swine News. 31:6.

Nemecheck, J.E., M.D. Tokach, S.S. Dritz, R.D. Goodband, J.M. DeRouchey, and J.C. Woodworth. 2016. Effects of diet form and corn particle size on growth performance and carcass characteristics of finishing pigs. Animal Feed Science and Technology. 214:136-141.

Miller, T. 2012. Swine Feed Efficiency: Influence of Pelleting. IPIC 25e.

Rochell, S.J., L.S. Alexander, R.D. Boyd, W.G. Van Alstine, J. Pettigrew, and R. Dilger. 2015. Effects of dietary soybean meal concentration on growth performance and immune response of pigs during a porcine reproductive and respiratory syndrome virus challenge. Journal of Animal Science 93:2987-2997.

Song, M., T.M. Che, Y. Liu, J.A. Soares, B.G. Harmon, and J.E. Pettigrew. 2012. Effects of dietary spray-dried egg on growth performance and health of weaned pigs. Journal of Animal Science 90:3080-3087.

Vansickle, J. 2011. Antibiotic-free pork production comes with a pretty high cost. National Hog Farmer. March. Can be found at: http://www.nationalhogfarmer.com/health-diseases/antibiotic-free-pork-production-cost-0315.

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. Midwest American Society of Animal Science Meetings, Des Moines, IA. Abstract #244.

A Nutritionist's Perspective on the Removal of Antibiotics in Poultry Production

Perspective d'un spécialiste en nutrition animale quant au retrait des antibiotiques en production avicole

Shivaram Rao
Senior Director of Nutrition and Technical Services
Pilgrim's Pride Corporation, 654 Univeter Road, Canton, GA 30105, USA

Abstract

Antibiotic free (ABF) poultry production is increasing due to consumer demand. The challenge for the commercial poultry nutritionists is to achieve the same end performance in a cost efficient manner under an ABF system as under regular production practices. A well planned ABF broiler production nutrition system should include preparatory steps such as maternal nutrition and *in ovo* interventions to improve viability of embryos and progeny as well as 18+ days of downtime between two successive broiler flocks. Maintaining the normal gut health of broilers through the grow-out is the nutritionists and veterinarians main goal. In this regard, the most challenging aspects are the control of coccidia and in turn prevention of necrotic enteritis. The long-term success and sustainability of any company's ABF program hinge upon the degree to which these issues are addressed. Other items to be considered to maintain normal gut health in the ABF program are a combination of prebiotics, probiotics, natural chemicals and risk management of mold toxins. Flock mortality may still spike up occasionally in which case nutritionists and veterinarians should have backup treatment plans ready.

Résumé

La production avicole sans antibiotiques augmente en raison de la demande des consommateurs. Le défi pour les spécialistes en nutrition des élevages avicoles commerciaux est de parvenir aux mêmes résultats finaux dans un système sans antibiotiques qu'avec les pratiques de production habituelles, sans compromettre la rentabilité. Un système de nutrition en production de poulets à griller sans antibiotiques bien planifié devrait comporter des étapes préparatoires, telles que l'alimentation des mères, des interventions *in ovo* visant à améliorer la viabilité des embryons et de la progéniture ainsi que des arrêts de production d'au moins 18 jours entre deux élevages successifs de poulets à griller. Maintenir un niveau de santé intestinale normal chez les poulets à griller pendant toute la période de production est le principal objectif des spécialistes en nutrition et des vétérinaires. Dans ce contexte, les éléments les plus complexes sont la lutte contre les coccidies et, donc, la prévention de l'entérite nécrotique. Le succès à long terme et la viabilité de tout programme de production sans antibiotiques reposent sur l'aptitude des producteurs à résoudre ces problèmes. Les autres aspects à considérer pour maintenir la santé intestinale des oiseaux élevés sans antibiotiques sont composés d'une combinaison de prébiotiques,

probiotiques, substances chimiques naturelles et gestion des risques de mycotoxines. Dans les troupeaux, la mortalité peut tout de même monter en flèche occasionnellement et, dans de tels cas, les spécialistes en nutrition et les vétérinaires doivent pouvoir recourir à des traitements d'urgence.

Introduction

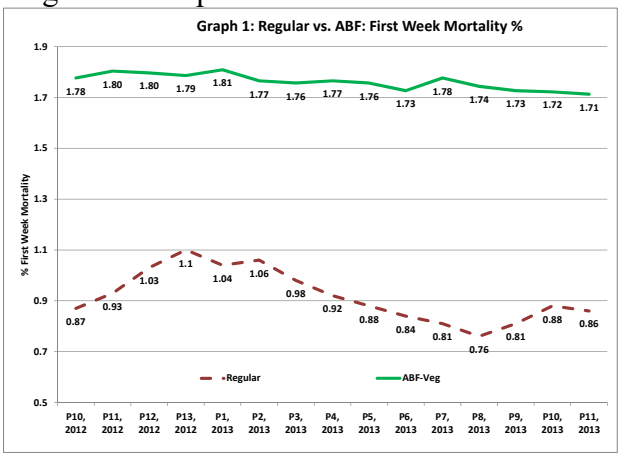
Based on a survey reported by Rennier, 12% of the US broiler-feed produced in 2015 was associated with "no antibiotics ever" programs (Rennier, 2016). Several major companies have antibiotic free (ABF) broilers in their portfolio at various levels. Two prominent examples being Purdue Farms' and Fieldale Farms, both of which are 100% ABF. These early adopters have proven that the system is a viable and sustainable model in the US food industry. Tyson Foods has announced its plans to reach complete ABF status in 2017. In 2016 Pilgrim's Pride added at least one million broilers per week to its already existing ABF capacity and in 2017 plans are underway to add another 2.5 million broilers per week to its ABF portfolio to reach over 25% of all of its broiler production under an ABF system. In anticipation of a significant shift in the market most commercial poultry companies in the US are developing several options for the future and expect their veterinarians, nutritionists, and managers to be up to date with the technical- /scientific developments and field observations occurring in the area of producing poultry in a totally antibiotic free system.

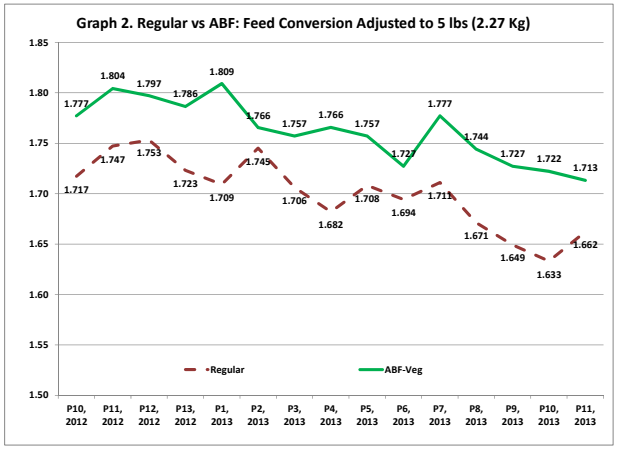
Most US companies are better prepared for ABF production**:** Use of antibiotics has been an established practice for several decades, however, many companies in the USA have been implementing the judicious use of antibiotics over the past ten years or so (Cervantes, 2015). Usually, feeds of several US broiler companies contain limited or even no antibiotics post 35 days of age even in their conventional program. This conservative use of antibiotics is enabled by improved management practices such as stricter adherence to longer downtimes (at least 14 days) between flocks, investing in broiler housing facilities with better ventilation, improved vaccination and health programs, and improved digestible efficiency of feed ingredients have enabled broilers to remain healthy under low antibiotic conditions. In a way, the US poultry industry has been inadvertently prepped for a successful transition into an ABF broiler production system. However, removing antibiotics completely is still a major challenge and the discussion below is focused on nutritional considerations to facilitate normal gut health.

Role of nutrition and gut health on the performance in poultry raised without antibiotics
Normal gut health is very important for cost efficient poultry production. Achieving normal gut health is relatively easier when a combination of coccdiostats and antibiotics are used. In the current system, nutritionists focus mainly on traditional nutrition work such as feed ingredient quality, nutrient specifications, ingredient costs, least cost formulation etc. However, to achieve normal gut health in an ABF system requires more involvement on the nutritionist's part. Nutritionists should work with veterinary groups and field management teams to understand and implement key processes to improve health in all vertical aspects of production such as growing healthy hen flocks to produce strong, clean hatching eggs, interventions during incubation in the hatchery to maintain good embryo health, sufficient downtime between successive broiler flock placements to reduce pathogenic stress, cocci control strategies, broiler house readiness, brooding, and nutritional strategies during hatching egg production as well as during embryo and

broiler grow-out. In other words, normal gut health in an ABF system is the result a holistic approach consisting of appropriate steps upstream and during production.

In the absence of antibiotics, highly complex microbiome management appear to be the key to achieving normal gut health and at present, our knowledge is very limited in poultry microbiome management. The decrease in broiler performance is significant due to disruption in microbiome damage caused by pathogenic microbes when companies switch their broiler program to ABF without streamlining the entire vertical process flow. In graph 1, monthly data over a year for the first-week mortality under a regular program with antibiotics use in the hatchery as well as in the feed is compared to a "no antibiotic ever" (NAE) program where antibiotic was not used in the hatchery or in the feed. The first-week broiler mortality increased from about 0.9% under the regular program to 1.75% range under the ABF program. The same complex also experienced a deterioration of about 6 points (Graph 2) in feed conversion ratio for a 5 lb. (2.27 Kg) broiler mainly due to necrotic enteritis which usually comes as a result of cocciodial originated lesions. The feed cost alone for producing live broiler increased by $0.05/lb ($0.11/kg). Other costs such as grower compensation increased as well.





Key nutritional opportunities (challenges) in antibiotic-free poultry production and possible solutions

As noted above, the performance could be off in an ABF program if it is not well thought out vertically and horizontally. The challenge for nutritionists is to achieve the same performance in an ABF system as in the conventional poultry grow-out in a cost efficient manner. The discussion below starts with the maternal (breeder hen flock) and *in-ovo* nutritional considerations within the hatchery even though hens and broiler embryo growth are outside of the ABF system. A sustainable, cost effective ABF program implementation will involve careful and well thought out process improvement steps from parents to progeny and throughout broiler grow-out.

1. Maternal nutrition to improve hatching egg quality and viability of chick embryo and progeny:
    a. Nutritional supplementation of Vitamin E, selenium, zinc, antioxidants, n-3 and n-6 fatty acids: In the designer egg production, nutritionists are able to increase the contents of vitamin E, zinc, and various other nutrients in the egg (Schideler et. al 2010). Similar approaches should be used to improve some key nutrients in

hatching eggs as well. Research has shown that just supplementing antioxidants such as ethoxyquin and BHT in feeds would increase vitamin E and selenium content of eggs. In addition, using higher levels of vitamin E, n-3 and n-6 fatty acids, selenium and zinc bound to organic molecules will help increase concentrations of these nutrients in egg and increases the possibility of the higher viability of embryo and progeny (Cherian, 2015; Surai, et al 2016).

b. Pre- and probiotics (direct fed microbial) should be used in feeds to help improve gut health and to reduce pathogenic microbes which in turn help produce cleaner eggs (Griggs and Jacob, 2005). Basic criteria to select pre- and probiotics are discussed later in this article.
c. Larger particle limestone usage in the feed would help improve egg shell quality and improve the livability of hens.
d. Feed digestibility can be improved by using exogenous enzymes (phytase, NSPase, proteases) to degrade anti-nutritional components in the feed. Use of phytase enzyme and slightly lowering added sodium content of feed will also help to keep the litter (bedding material) drier and thus help egg quality.
e. Biotransformation of mold toxins is an exciting concept and an investment in this technology to manage mold toxin risk makes sense for parents and grandparent flocks. This concept uses enzymes, microbial products, and natural plant products to transform mold toxins and render them harmless to poultry.

Maternal feeding to improve embryo and progeny health and performance is a very economical way and nutritionists should explore this possibility in their ABF system.

2. In-ovo nutrient delivery during embryo growth in the hatchery: This technique could be used to improve immune functions (Dibner et al. 2008) of broiler chicks. Uni (2014) outlined several nutritional candidates for in ovo feeding that can improve gut health, early growth, improved performance, and meat yield. Although this is not commonly used at present, nutritionists should be exploring possible to identify cost-effective nutritional candidates for this route of delivery to improve gut health and early growth.

3. Coccidia control and prevention of necrotic enteritis is the most important objective in achieving normal gut health in ABF broiler production (Cervantes, 2015). However, various possible options available to achieve this goal are not directly discussed in this article because it is covered in detail elsewhere in this symposium. Even so, during the discussion of other options- /agents available to achieve normal gut health, it will be noted if they have any side benefits in controlling cocci and necrotic enteritis.

4. Early nutrition (day 1 to the first 7 to 10 days) of broilers:
    a. With antibiotics and coccdiostats being restricted (excluding some ABF programs which allow ionophores to control coccidia), nutritionists and veterinarians are exploring combinations of prebiotics, probiotics, and plant-derived antimicrobial feed additives. The strategy should be a) to reduce pathogenic microbes, b) to improve food safety (lower salmonella, campylobacter) and if possible c) to improve broiler performance as well. With limited money available to spend on feed, it is difficult to achieve all those goals listed under the strategies. Also, with so many vendors and options available, this

author recommends working with vendors who not only have solid research backed, consistent product(s) but have strong technical teams that understand the science to support and customize their products and techniques if necessary as the GI tract microbiome is a constantly evolving and moving target.

b. We should make sure that feed is highly digestible, pelleted and crumbled optimally. Use of exogenous enzymes targeted to soybean meal, phytase, and NSPases and are more appropriate at this stage.

c. Nutritionists should utilize super-dosing of phytase enzyme in the feed to degrade as much anti-nutritional phytic acid as possible. This consequently leads to less mucin production which then may in turn help reduce necrotic enteritis. A combination of lower "added" calcium with super-dosing of phytase to release plant bound calcium can help lower NE and improve broiler performance (Paiva, et al., 2013).

d. A pre-starter feed could be designed for this phase. Use of synthetic amino acids is highly encouraged to prevent excess protein in the feed which helps to prevent excess ammonia build up in the facility and also provides fewer nutrients in the hind gut for pathogenic microbes.

e. Risk management of mold toxins should be a part of the strategy as well. If toxin binders are used, be cautious to avoid toxin binders that can bind to expensive organic minerals present in the trace mineral premix. Biotransformation of mold toxins may be an option for some ABF systems.

5. Avoid nutrition related stress during peak cocci cycling which under cocci-vaccination could occur between 14 to 20 days of age. It would be preferable to avoid switching from a crumbled first feed to pelleted second feed during this stressful period. A failure to execute a good cocci control program would lead to necrotic enteritis at this stage or during the third week in broilers. The prebiotic, probiotic and natural antimicrobial feed additive program started in the first feed should be continued in the subsequent feeds but dosage could be lowered if internal research results lead in that direction.

6. Nutritionists should keep exploring natural or synthetic chemicals options that could be beneficial in improving gut health. For example, supplementing feeds with natural betaine may help reduce or moderate cocci lesions and improve performance (Amerah & Ravidnran, 2015).

7. Even with all the extensive planning and its execution, broilers may still occasionally have severe spikes in mortality. Therefore, it is essential to have treatment options available when early signs of increased mortality are observed. One popular option is acidified copper sulfate administered through drinking water. However, it would be desirable to have two or three such effective options readily available with written treatment protocol as well

as access to the treatment material and a field team prepared to execute the treatment plan if necessary.

References

Amerah, A.M., and Ravindran, V. 2015. Effect of coccidia challenge and natural betaine supplementation on performance, nutrient utilization, and intestinal lesion scores of broiler chickens fed suboptimal level of dietary methionine. Poult Sci. 94(4):673-80

Cerveantes, H.M. 2015. Antibiotic-free poultry production: Is it sustainable? J. Appl. Poult. Res. 24 (1):91-97

Cherian, G. 2015. Nutrition and metabolism in poultry: the role of lipids in the early diet. J. Anim. Sci. & Biotechnology, 6:28.

Dibner, J. J, Richards, J. D., Knight, C. D. 2008. Microbial imprinting in gut development and health. J. Appl Poult. Res. 17:174-188

Griggs, J. P., and J. P. Jacob. 2005. Alternatives to antibiotics for organic poultry production. J. Appl. Poult. Res. 4:750-756

Lokesh E., Dhinesh Kumar R, Bhanuprakash V, Shilpa Choudhary, Nirmala Muwel, Kanti Raje, and Mokshata Gupta. In ovo Ealry feeding for advanced feed utilization in chicks. International Journal of Science, Environment and Technology, 2017 6(1) 560-565

Paiva, D. M., Walk, C. L., and McElroy, A.P., 2013. Influence of dietary calcium level, calcium source, and phytase on bird performance and mineral digestibility during a natural necrotic enteritis episode. Poult. Sci. 92(12): 3125-3133

Rennier, G. 2016. Fast growth seen for ABF poultry production, but for how long? Poultry Health Today. Available at: http://poultryhealthtoday.com/fast-growth-seen-for-abf-poultry-production-but-for-how-long/

Schedeler, S. E., P. Weber and D. Monsalve. 2010. Supplemental vitamin E and selenium effects on egg production, egg quality, and egg deposition of alpha-tocopherol and selenium. J. Appl Poul Res. 19 (4) 354-360
Surai, P., V. Fisnin and F. Karadas. 2016. Antioxidant systems in chick embryo development. Part 1. Vitamin E, carotenoids and selenium. Animal Nutrition. 2(1):1-11

Tyson Foods website 2017. Available at: http://www.tysonfoods.com/media/position-statements/antibiotic-use.aspx

Uni, Z. 2014. The effects of *in ovo* feeding. 2014. Arkansas Nutrition Conference, Rogers, Arkansas. 9 pages. Available at: http://www.thepoultryfederation.com/public/userfiles/files/Zehava%20Uni%20Presentation.pdf

Raising Healthy Birds without Antibiotics

Élever des oiseaux en santé sans antibiotiques

Scott J. Gustin, DVM, MAM
Director of Veterinary Services, Domestic Poultry, Tyson Foods, Inc., SPRINGDALE, AR 72762
scott.gustin@tyson.com

Abstract

Recent consumer and food service demand for broilers raised without antibiotics has led to dramatic changes in how gut health is managed. In the past, ionophores and antibiotics with anti-Clostridial effects were used to compliment the need in controlling coccidiosis and Necrotic Enteritis (NE). In the "No Antibiotics Ever (NAE)" arena, coccidiosis must be controlled through traditional anti-protozoal products or coccidiosis immunization. However, success will be determined by finding products to provide anti-Clostridial effects or otherwise positively influence microflora balance. The most commonly used antibiotic alternative products in NAE production usually fall in the categories of probiotics, prebiotics, phytogenics/essential oils, or organic acids. Success in NAE production will likely involve a combination of these alternative products along with managing the ever present *Eimeria* spp. to achieve satisfactory feed conversion and mortality.

Résumé

L'intérêt exprimé depuis peu par les consommateurs et les services alimentaires pour les poulets à griller élevés sans antibiotiques a entraîné des bouleversements dans la façon de gérer la santé intestinale. Auparavant, des ionophores et antibiotiques doués d'effets anticlostridiens étaient utilisés pour aider à maîtriser la coccidiose et l'entérite nécrotique. Dans ce contexte de production sans antibiotiques, la coccidiose doit être combattue avec des produits classiques contre les protozoaires ou par le biais de la vaccination. Quoi qu'il en soit, le succès passera par la découverte de produits exerçant un effet anticlostridien ou qui, d'une façon ou d'une autre, influenceront positivement l'équilibre de la microflore. Les produits de substitution les plus souvent utilisés en production sans antibiotiques appartiennent généralement aux catégories des probiotiques, des prébiotiques, des huiles phytogènes ou essentielles ou des acides organiques. Le succès des productions sans antibiotiques viendra fort probablement de l'utilisation d'une combinaison de ces produits de remplacement et d'une bonne gestion des omniprésentes espèces d'*Eimeria* permettant d'atteindre des valeurs d'efficacité alimentaire et de taux de mortalité acceptables.

Introduction

Antibiotic free production, in contrast to conventional production with some prophylactic antibiotic use, necessitates a keen re-evaluation of chick quality and gut health. Chick quality issues will more common result from gram negative organisms and

recontamination issues through in-ovo vaccination. Attention to breeder farm hygiene, nest pad maintenance, and farm grading and storage are imperative to improving chick quality. On the hatchery side, hatch window timing and incubation conditions that favor navel closure are critical. Continuous attention to chick quality is even more paramount in producing healthy antibiotic-free broilers.

The key determinant of success in antibiotic free broilers is control of Necrotic Enteritis (NE). While other endemic diseases may still remain, NE is the primary disease of increased incidence during this transition. Control of coccidiosis in an NAE program is done via anti-protozoal products or vaccines for the prevention of coccidiosis. Both intervention strategies have their shortcomings, but in the experience of the author, coccidiosis vaccines have a much greater preponderance for NE breaks that exceed a tolerable threshold for mortality that would meet a proposed NAE audit scheme. The key to success in NAE production is finding products and strategies that complement previously known successful programs in coccidiosis and *Clostridium perfringens* (CP) control.

Mechanisms and Decision Making

Controlling NE requires products that can ameliorate either coccidiosis or Clostridium perfringens. The newest range of products on the market fulfilling the void of antibiotics are probiotics, prebiotics, phytogenics/essential oils, and organic acids. The difficulty that is presented is evaluating the products in a research or field setting as virtually all products do not have true health claims and are sold as "flavouring agents." As a result, there are several key components in dealing with NAE product evaluation:

1. Understanding modes of action and complementing roles
2. Research and field trials
3. Defining "success"
4. Cost vs. benefit analysis

Gut health antibiotic alternatives have a variety of modes of action against *Clostridium* spp, *Eimeria* spp, and protective or flora modulating effects. Both single-strain and multi-strain probiotics have been proven to reduce NE by reduction of intestinal CP levels (Jeong, 2014 and Mohnl 2010.) Additionally, prebiotic products have been demonstrated to have indirect and direct effects on binding pathogenic bacteria and supporting the growth of beneficial bacteria, adding an additional pathway to combat NE and other conditions. These products have been readily available for over 10 years, and have found their value and niche within conventional and NAE programs.

Perhaps the greatest increase in Gut health antibiotic alternative product offerings in the past 5 years has been in the field of organic acids and phytogenic/botanical/EO products. Botanical extracts alone or in combination with organic acids have shown to improve intestinal integrity and control CP-induced NE (Grilli 2015, Lee 2013, Timbermont 2010.) These products appear to not only have anti-clostridial effects, but additionally anti-protazoal shedding effects in the case of certain compounds. But compared to probiotic and prebiotic compounds, botanicals for the most part carry a higher price per ton and do not offer any additional claims of efficacy given their approval status.

Therein lies the greatest struggle of NAE production: understanding your gaps in protection against NE and determining the most cost effective means of achieving this under research and field conditions. Retrofitting existing in-house research facilities to reflect field conditions and NAE programs must be done quickly and accurately. Coccidiosis products and management strategies in such facilities should emulate a level of coccidiosis and NE challenge that will be observed in the field. There is also a wide chasm between the needed speed of discovery and the availability of trial space in commercial and University settings and turnaround of results. Field studies, although fraught with variabilities in season and other program changes, should be structured so that, at minimum, tracking mechanisms are in place to determine when products reach the field and are withdrawn. Size, scale, and replicates, are distinct advantages if one has the discipline to stick with programs over growout cycles without incessant adjustment of product inclusions and replacements.

Although cost is the primary driver for any integrated operation, additional factors have impacted the relative "success" of NAE programs. Consideration must be given for the cost of removal of a house or flock from and NAE program and how this flock will be relegated to a different product stream within a processing plant and retail or food service distribution. Participating companies often have a mortality/morbidity threshold for treatment with an antibiotic therapy when palliative or therapeutic antibiotic alternative products have failed. Reaching this "threshold" in some locations may greatly influence the willingness to evaluate and purchase certain alternative products. So while cost is always a consideration, the game has certainly changed in terms of value and preventative efforts that will be employed to avoid product segregation down the line. Using all the available tools and strategies that exist today, reaching mortality and performance levels of conventional broiler production is certainly possible for many operations and presents a new challenge for those that wish to provide a product the customer desires.

References

Grilli, E., B. Tugnoli, J. Passey, C. Stahl, A. Piva, A. Moeser. 2015. Impact of dietary organic acids and botanicals on intestinal integrity and inflammation in weaned pigs. BMC Vet Res 11:96

Jeong, J.S., I. Kim. 2014. Effect of *Bacillus subtilis* C-3102 spores as a probiotic feed supplement on growth performance, noxious gas emission, and intestinal microflora in broilers. Poult Sci 93 (12): 3097-3103

Lee, S. H., H. Lillehoj, S. Jang, E. Lillehoj, W. Min, D. Bravo. 2013. Dietary Supplementation of young broiler chickens with Capsicum and turmeric oleoresins increases resistance to necrotic enteritis. Brit J Nutr. 110 (5): 1-8

Mohnl, M. 2010 Inhibition of Clostridium Perfringens through Probiotic Strains and Efficacy of Mulispecies Probiotic to Reduce NE in Poultry. Avian Pathology S20.

Timbermont, L., A. Lanckriet, J. Dewulf, N. Nollet, K. Schwarzer, F. Haesebrouck, R. Ducatelle, F. Van Immerseel. 2010. Control of Clostridium perfringens-induced necrotic enteritis in broilers by target released butyric acid, fatty acids, and essential oils. Avian Path. Vol 39 : 117-121

Conference

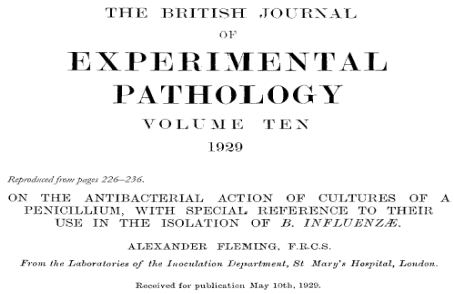
Colloque

Sponsor / *Commanditaire*:

Antimicrobial consumption and resistance – a One Health view

Ramanan Laxminarayan
2017 ANCC Conference

I. Drug resistance is rising worldwide and threatens gains made in reducing the burden of infectious diseases



THE BRITISH JOURNAL
OF
EXPERIMENTAL
PATHOLOGY
VOLUME TEN
1929

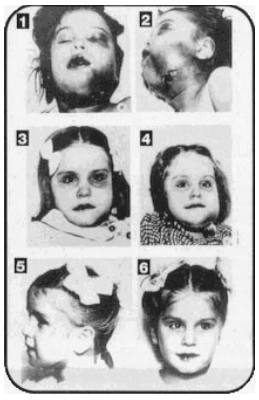
Reproduced from pages 226–236.

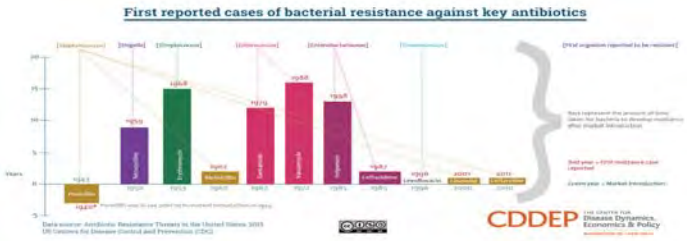
ON THE ANTIBACTERIAL ACTION OF CULTURES OF A PENICILLIUM, WITH SPECIAL REFERENCE TO THEIR USE IN THE ISOLATION OF *B. INFLUENZÆ*.

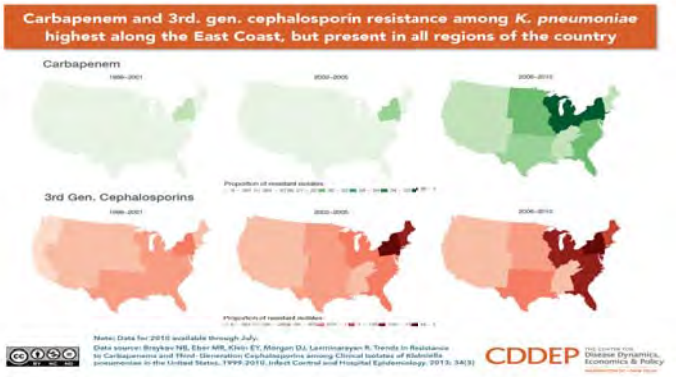
ALEXANDER FLEMING, F.R.C.S.

From the Laboratories of the Inoculation Department, St Mary's Hospital, London.

Received for publication May 10th, 1929.

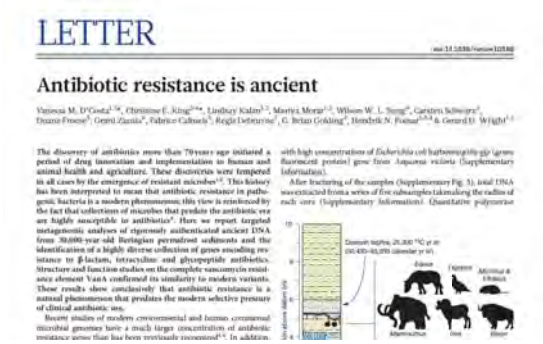




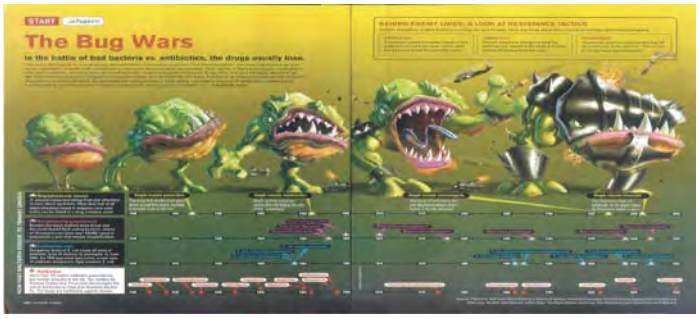


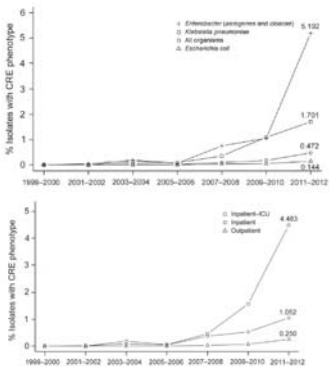
It is not difficult to make microbes resistant to penicillin in the laboratory by exposing them to concentrations not sufficient to kill them, and the same thing has occasionally happened in the body…

Alexander Fleming, 1945



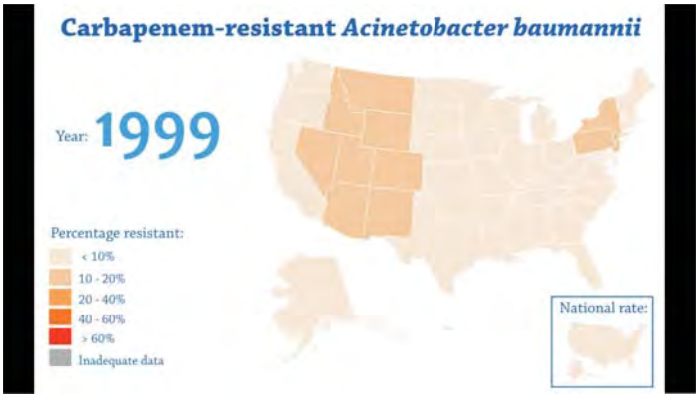
D'Costa, Nature, 2011



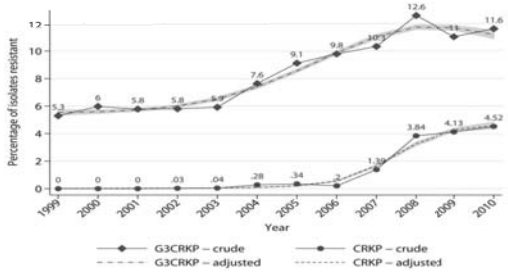


CRE rates in children grew between 2000 and 2012

Logan et al, EID, 2015

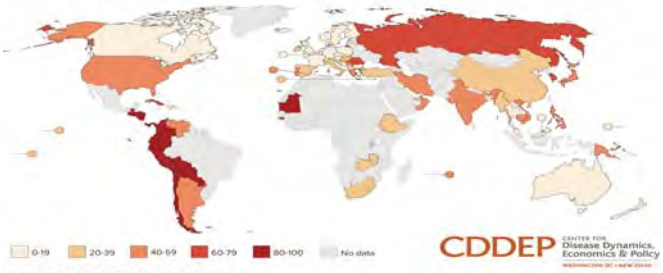


Increasing *Klebsiella pneumoniae* resistance to carbapenems (CRKP) and third-generation c



Braykov et al ICHE, 2012

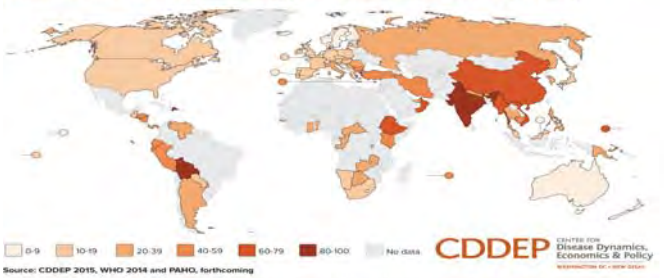
Percentage of *Staphylococcus aureus* that are methicillin resistant (MRSA), by country (most recent year, 2011-14)



Source: CDDEP 2015, WHO 2014 and PAHO, forthcoming

Where available, data from hospital-associated MRSA and invasive isolates have been used. In their absence, data from community-associated MRSA or all specimen sources are included. Only countries that reported data for at least 30 isolates are shown. Depending on the country, resistance to one or more of the following drugs were used to test for MRSA: Oxacillin, cefoxitin, flucloxacillin, cloxacillin, dicloxacillin, and methicillin. Intermediate-resistant isolates are included as resistant in some calculations, as in the original data source.

Percentage of extended-spectrum beta-lactamase producing *Escherichia coli, by country (most recent year, 2011-2014)**

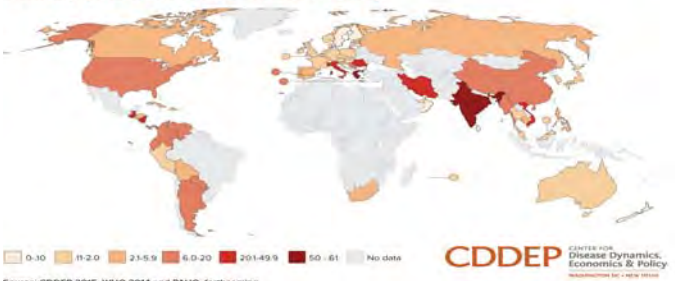


Source: CDDEP 2015, WHO 2014 and PAHO, forthcoming

Where available, data from invasive isolates have been used. In their absence, data from all specimen sources are included. Only countries that reported data for at least 30 isolates are shown. Depending on the country, resistance to one or more of the following drugs were used: Ceftazidime, ceftriaxone and cefotaxime. Intermediate-resistant isolates are included as resistant in some calculations, as in the original data source.

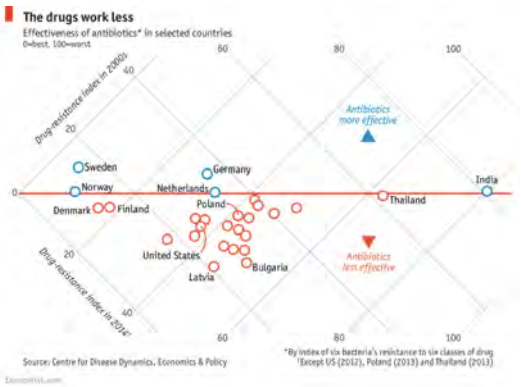
*Indicated by third-generation cephalosporin resistance.

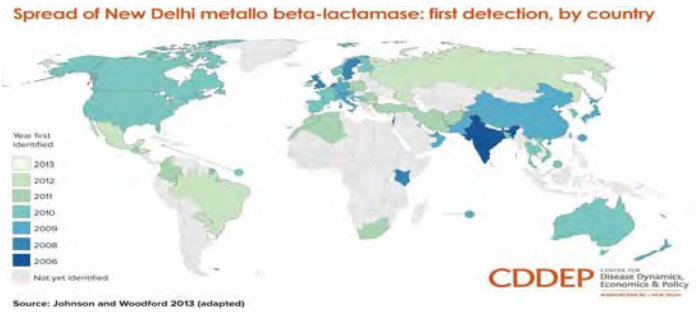
Percentage of carbapenem-resistant *Klebsiella pneumoniae*, by country (most recent year, 2011-2014)



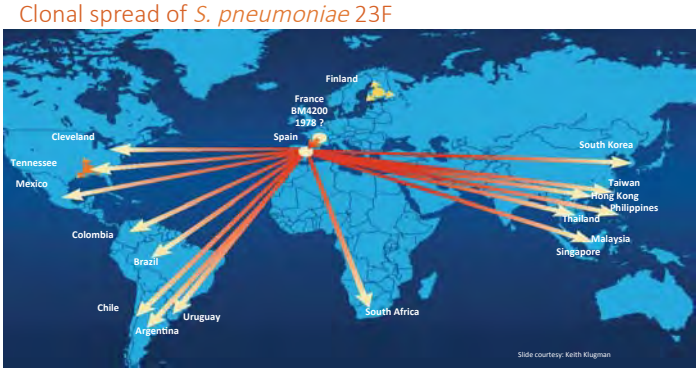
Source: CDDEP 2015, WHO 2014 and PAHO, forthcoming

Where available, data from invasive isolates have been used. In their absence, data from all specimen sources are included. Only countries that reported data for at least 30 isolates are shown. Depending on the country, resistance to one or more of the following drugs were used: imipenem, meropenem, ertapenem and doripenem. Intermediate-resistant isolates are included as resistant in some calculations, as in the original data source.

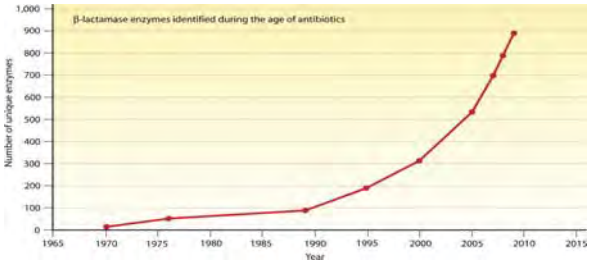




Clonal spread of *S. pneumoniae* 23F

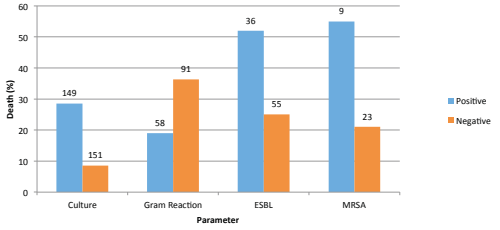


Numbers of unique β-lactamase enzymes identified since introduction of first β-lactam antibiotics



Davies and Davies, Microbiol. Mol. Biol. Rev. 2010.

Mortality outcomes are worse in neonates with resistant infections



Kayange M, Kamugisha E, Mwizamholya DL, Jeremiah S, Mshana SE. 2010. Predictors of positive blood culture and deaths among neonates with suspected neonatal sepsis in a tertiary hospital, Mwanza- Tanzania. BMC Pediatrics 10: 39.

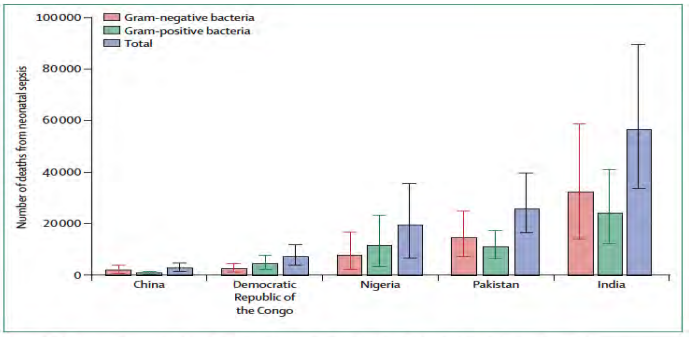
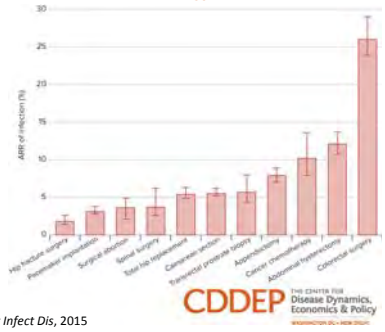


Figure 2: **Estimated neonatal sepsis deaths caused by bacteria resistant to first-line antibiotics in five high-burden countries**

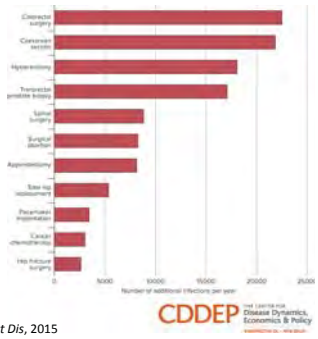
Laxminarayan et al Lancet, 2015

Absolute risk reduction (ARR) of infection with antibiotic prophylaxis in common surgical procedures and blood cancer chemotherapy in the USA



Teillant et al, *Lancet Infect Dis*, 2015

Number of additional infections per year in the USA under a 30% decreased efficacy of antibiotic prophylaxis



Teillant et al, *Lancet Infect Dis*, 2015

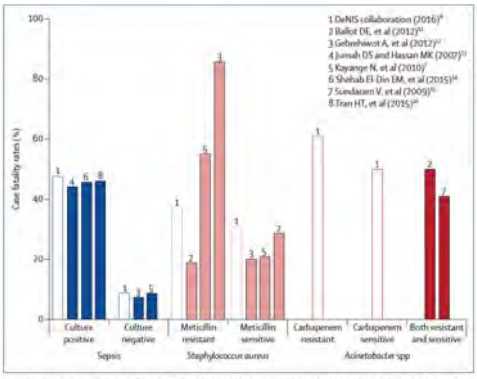
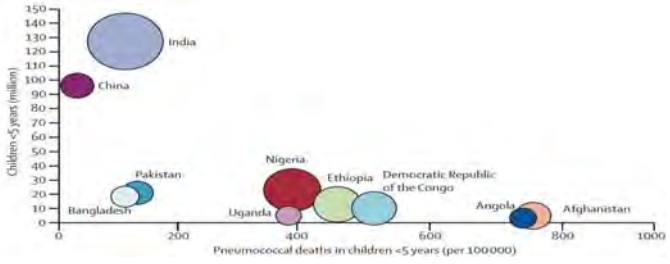


Figure: Case fatality rates from the DeNIS study (unshaded) compared with earlier studies (in solid colours)

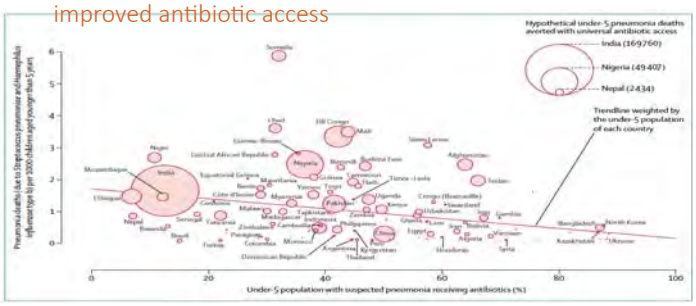
II. Rising incomes and increasing access to antibiotics are saving lives (although lack of access still kills more people than antibiotic resistance) but are not a good substitute for public health

Bacterial diseases are still major killers in developing countries because of lack of access to antibiotics



O'Brien et al, Lancet 2009

Pneumococcal pneumonia deaths avertable with improved antibiotic access

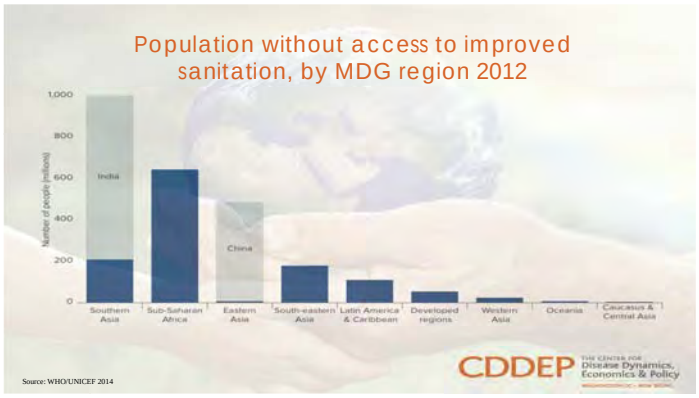


Laxminarayan et al, Lancet, 2015

What are we asking of antibiotics?

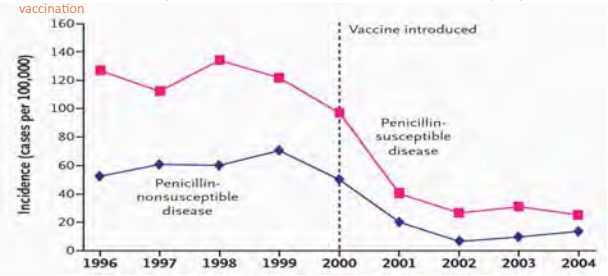
Substitute for immunization, infection control and water/sanitation

Substitute for immunization, infection control and water/sanitation



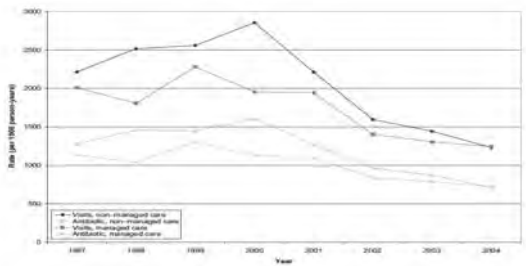
Vaccines can be effective

Invasive disease caused by Pneumococci in children under two declined in the US post pneumo vaccination



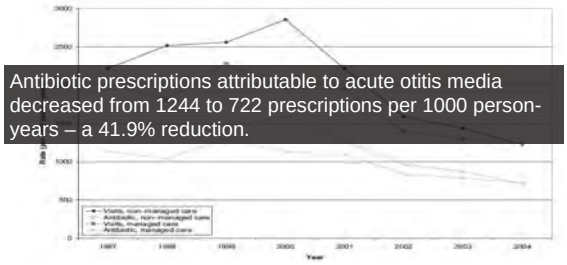
Kyaw MH et al. N Engl J Med 2006;354:1455-1463.

Effect of PCV7 introduction in 2000 on antibiotic prescriptions and ambulatory care visits



Zhou et al, Pediatrics 2008

Effect of PCV7 introduction in 2000 on antibiotic prescriptions and ambulatory care visits



Antibiotic prescriptions attributable to acute otitis media decreased from 1244 to 722 prescriptions per 1000 person-years – a 41.9% reduction.

Zhou et al, Pediatrics 2008

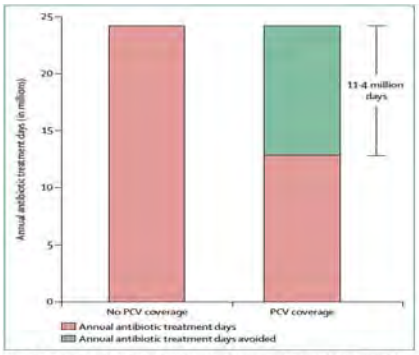
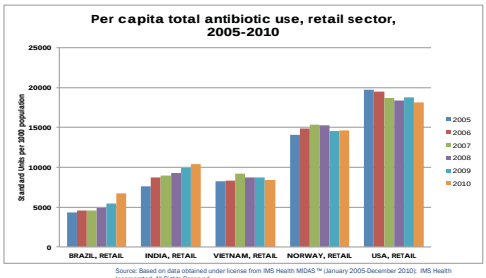


Figure 3: Days on antibiotics for suspected pneumonia, averted by provision of pneumococcal conjugate vaccine (PCV)
Bar represents antibiotic days avoided with PCV coverage.

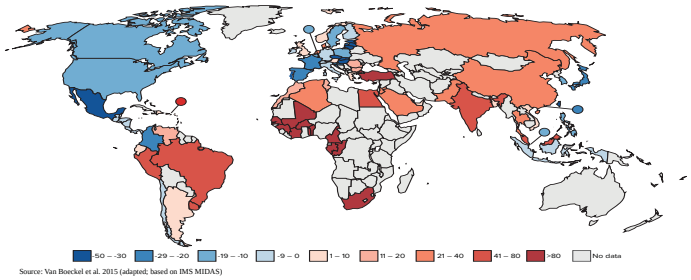
Laxminarayan et al Lancet, 2015

Antibiotic consumption is increasing in developing countries...



Source: Based on data obtained under license from IMS Health MIDAS ™ (January 2005-December 2010); IMS Health Incorporated. All Rights Reserved.

Percentage change in antibiotic consumption per capita 2000–2010*, by country

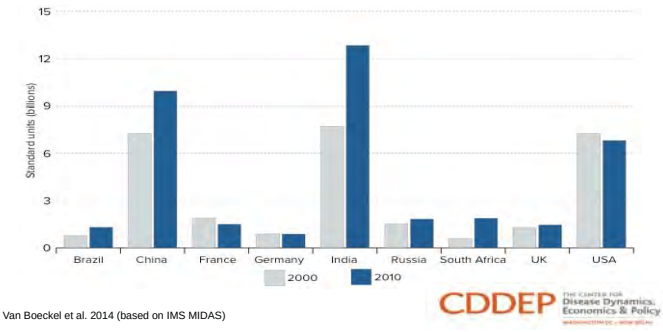


Source: Van Boeckel et al. 2015 (adapted; based on IMS MIDAS)

*Data for Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua and Panama were available only as a group classified as Central America. Similarly, data for Benin, Burkina Faso, Cameroon, Côte d'Ivoire, Gabon, Guinea, Mali, Republic of the Congo, Senegal, and Togo were grouped and classified as French West Africa. The data for these countries represent the estimates for the corresponding regional groupings they belong to. For countries that did not have data available for 2000, the values for the earliest years for which data were available after 2000 were used to calculate the percentage changes. These countries and initial years are Algeria (2002), Bangladesh (2007), Croatia (2005), Netherlands (2005), and Vietnam (2005).

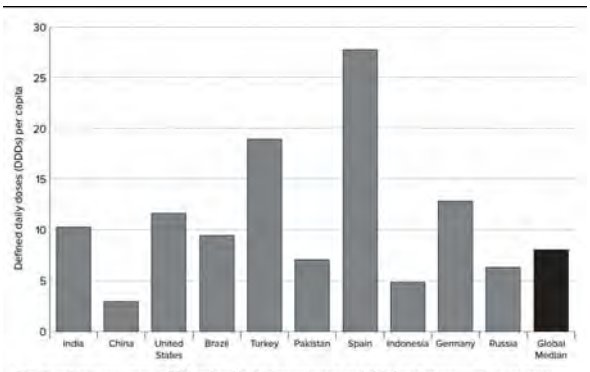
CDDEP The Center for Disease Dynamics, Economics & Policy

Total antibiotic consumption in selected countries, 2000 and 2010

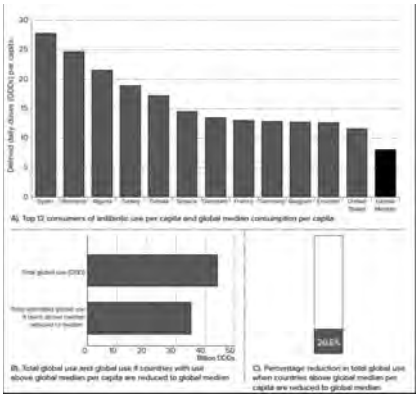


Van Boeckel et al. 2014 (based on IMS MIDAS)

CDDEP The Center for Disease Dynamics, Economics & Policy



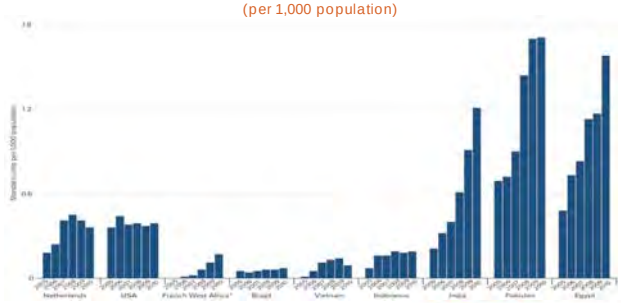
Top 10 antibiotic consumers in 2014, ordered by total consumption, and global median consumption per capita.



Antibiotic use per capita by income in selected countries, 2010

Source: Van Boeckel et al. 2014 (based on IMS MIDAS) and World Bank 2015

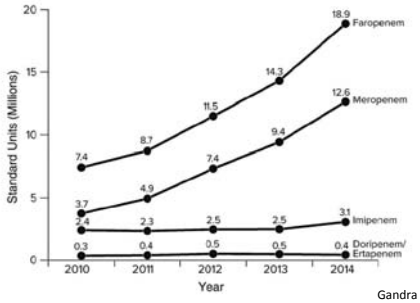
Carbapenem retail sales in selected countries, 2005–2010
(per 1,000 population)



Source: Laxminarayan et al. 2013 (based on IMS MIDAS)

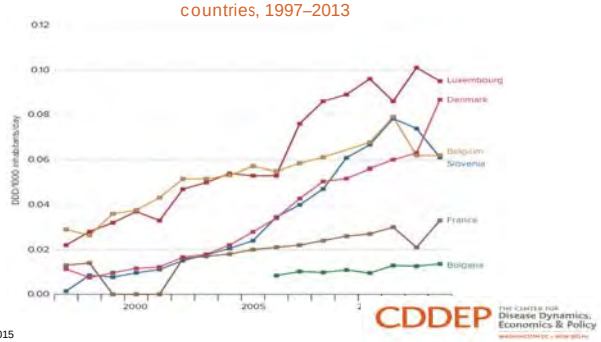
*An IMS grouping of Benin, Burkina Faso, Cameroon, Côte d'Ivoire, Gabon, Guinea, Mali, Republic of the Congo, Senegal, and Togo

Faropenem consumption has increased by 154% since it was approved for use in India in 2010



Gandra et al, Clin Inf Dis, 2016

Carbapenem consumption in the hospital sector in selected European countries, 1997–2013



ESAC-Net 2015

Non-prescription use of antimicrobials is common

Figure 2: Frequency of non-prescription use of antimicrobials in the general population based on published works

Morgan et al, Lancet ID, 2011

Table 1. Workforce of Doctors and Nurses According to Country or Region in 2010.*

Country or Region	Population	Doctors	Nurses	Doctors and Nurses/ 1000 Population	Nurse-to-Doctor Ratio
	in millions	*in thousands*			
Country					
China	1338	1915	1,864	2.8	0.97
India	1225	768	1,179	1.6	1.54
United States	309	756	3,064	12.3	4.05
Brazil	195	338	1,278	8.3	3.78
United Kingdom	62	166	626	12.7	3.77
South Africa	50	37	198	4.7	5.30
Region					
Americas	937	1974	4,947	7.4	2.5
Europe	899	2744	5,870	9.6	2.1
Middle East and North Africa	590	654	894	2.6	1.4
Southeast Asia	1795	997	1,810	1.6	1.8
Sub-Saharan Africa	847	150	778	1.1	5.2
Western Pacific	1821	2696	3,814	3.6	1.4
World	6888	9216	18,114	4.0	2.0

* A doctor or nurse is defined as a person with the appropriate qualifications recognized in his or her own country. In this table, the nurse workforce includes nurses and midwives. Data are from the World Health Organization.*

Table 1. Workforce of Doctors and Nurses According to Country or Region in 2010.*

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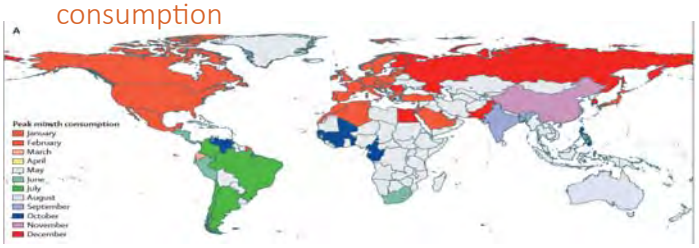
United States	309	756	3,064	12.3	4.05
Brazil	195	338	1,278	8.3	3.78
United Kingdom	62	166	626	12.7	3.77
South Africa	50	37	198	4.7	5.30
Region					
Americas	937	1974	4,947	7.4	2.5
Europe	899	2744	5,870	9.6	2.1
Middle East and North Africa	590	654	894	2.6	1.4
Southeast Asia	1795	997	1,810	1.6	1.8
Sub-Saharan Africa	847	150	778	1.1	5.2
Western Pacific	1821	2696	3,814	3.6	1.4
World	6888	9216	18,114	4.0	2.0

* A doctor or nurse is defined as a person with the appropriate qualifications recognized in his or her own country. In this table, the nurse workforce includes nurses and midwives. Data are from the World Health Organization.*

The flu season is a key driver of antibiotic consumption



Van Boeckel et al, Lancet Inf Dis, 2014

Influenza in the United States is nearly perfectly predicted by antibiotic sales data

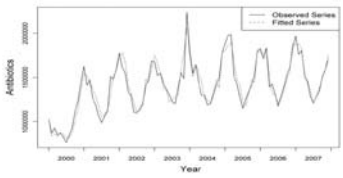
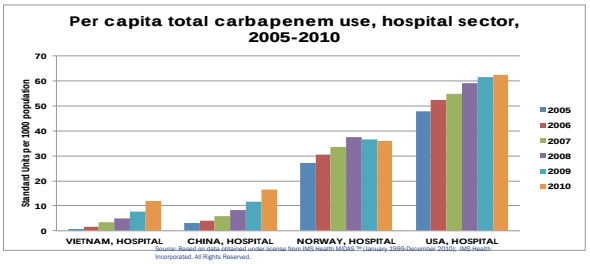
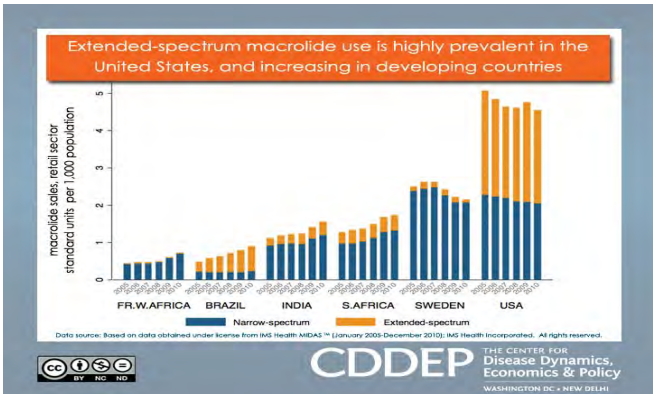


FIGURE 1. Observed and fitted antibiotics series from 2000 to 2007. The solid line represents the actually observed antibiotics series; the dashed line represents the fitted antibiotics series from the time series regression model that uses influenza-like illness as an explanatory series.

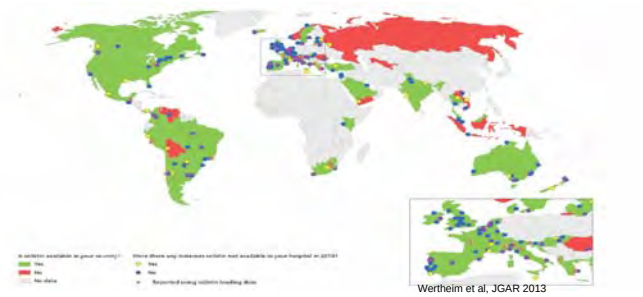
Polgreen et al Inf Cont Hosp Epi, 2011

Hospital use of carbapenems is rapidly growing





Global availability of colistin



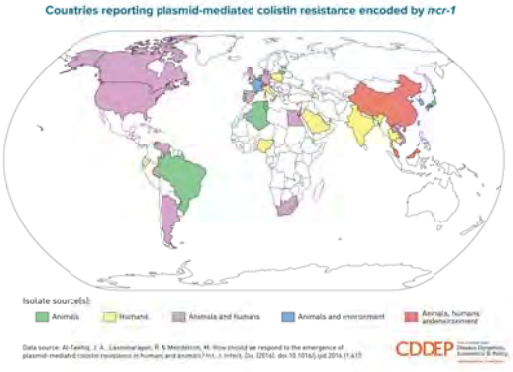
Wertheim et al, JGAR 2013

Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study

Yi-Yun Liu*, Yang Wang*, Timothy R Walsh, Ling-Xian Yi, Rong Zhang, James Spencer, Yohei Doi, Guobao Tian, Baolin Dong, Xianhui Huang, Lin-Feng Yu, Dianxin Gu, Hongwei Ren, Xiaojie Chen, Lucheng Lv, Dandan He, Hongwei Zhou, Zisen Liang, Jian-Hua Liu, Jianzhong Shen

Summary

Background Until now, polymyxin resistance has involved chromosomal mutations but has never been reported via horizontal gene transfer. During a routine surveillance project on antimicrobial resistance in commensal *Escherichia coli* from food animals in China, a major increase of colistin resistance was observed. When an *E coli* strain, SHP45, possessing colistin resistance that could be transferred to another strain, was isolated from a pig, we conducted further analysis of possible plasmid-mediated polymyxin resistance. Herein, we report the emergence of the first plasmid-mediated polymyxin resistance mechanism, MCR-1, in Enterobacteriaceae.



Dealing with resistance

Make better use of existing drugs

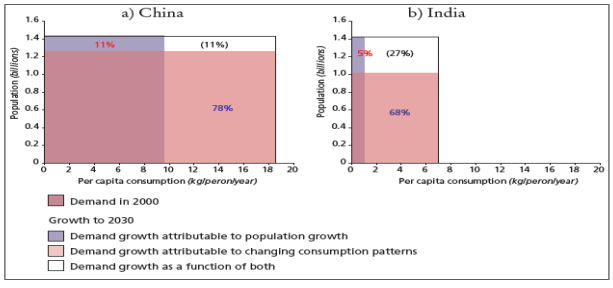
Find new drugs

III. Antibiotic use in animal sector is increasing globally in response to the tremendous growth in demand for animal protein. Meanwhile antibiotic manufacturing is altering the resistome in the environment.

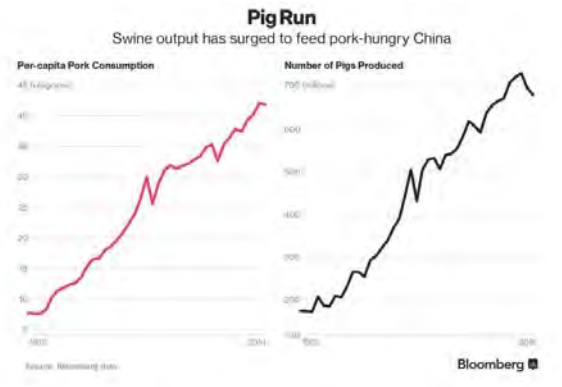
Antibiotic use for growth promotion and disease prevention

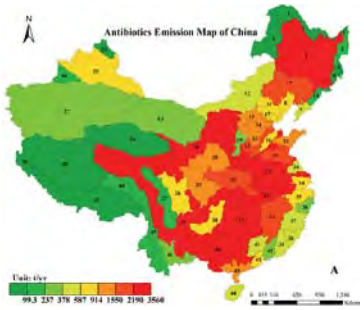
2/3rds of the tonnage of antibiotics sold worldwide are used in agriculture

Demand for poultry in India and China is set to increase two to seven fold between 2000 and 2030



FAO, 2011





- Total consumption in China - 92700 tons in 2013,
- 54000 tons of antibiotics excreted by human and animals - much of this entered into the receiving environment following various wastewater treatments into 58 river basins of China

Zhang et al, Env Sci Tech, 2015

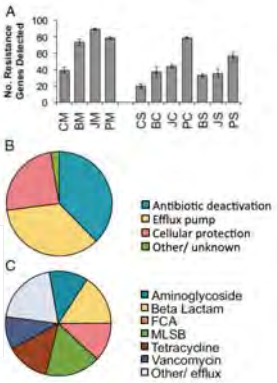
PNAS

Diverse and abundant antibiot in Chinese swine farms

Yong-Guan Zhu[a,b,1,2], Timothy A. Johnson[c,d,1], Jian-Qiang Su[a], Min
Syed A. Hashsham[c,e], and James M. Tiedje[c,d,2]

[a]Key Lab of Urban Environment and Health, Institute of Urban Environment, Chinese A
Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China; and [c]Ce
Sciences, and [d]Civil and Environmental Engineering, Michigan State University, East L

Contributed by James M. Tiedje, December 31, 2012 (sent for review October 31, 201



High-capacity quantitative PCR arrays detected 149 unique resistance genes among all of the farm samples, the top 63 ARGs being enriched 192-fold (median) up to 28,000-fold (maximum) compared with their respective antibiotic-free manure or soil controls.

Pharmaceuticals and Personal Care Products in the Environment

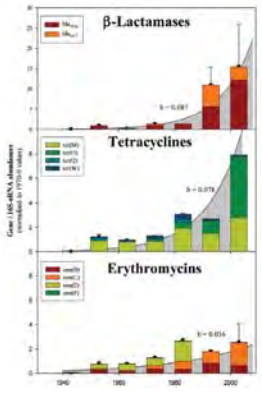
CONTAMINATION OF SURFACE, GROUND, AND DRINKING WATER FROM PHARMACEUTICAL PRODUCTION

JERKER FICK,*† HANNA SÖDERSTRÖM,† RICHARD H. LINDBERG,† CHAU PHAN,† MATS TYSKLIND,† AND D.G. JOAKIM LARSSON‡
†Department of Chemistry, Umeå University, Linnaeusväg 6, SE-90187 Umeå, Sweden

High amounts of four antibiotics were measured in the lakes that do not take in wastewater from the sewage plant. The levels of ciprofloxacin (2.5 mg/L) and cetirizine (20 µg/L) in one of the lakes was higher than previously measured levels in the blood of people taking the medications, report the authors. This suggests there are other unknown sources – perhaps illegal dumping – of wastewater responsible for polluting the lakes.

In addition, effluents from a wastewater treatment had concentrations of ciprofloxin of 14 milligrams per liter (mg/L) and cetirizine as high as 1.2 mg/L. These concentrations are approaching therapeutic doses (concentrations that would kill some microorganisms outright). Concentration reported in the US range in the nanograms per liter (ng/L), which are one million fold less.

Fick et al Env Tox and Chem, 2009



Increase of antibiotic resistance genes among soils collected at five sites in The Netherlands from 1940 to 2008.

Knapp et al Env Sci Tech, 2010

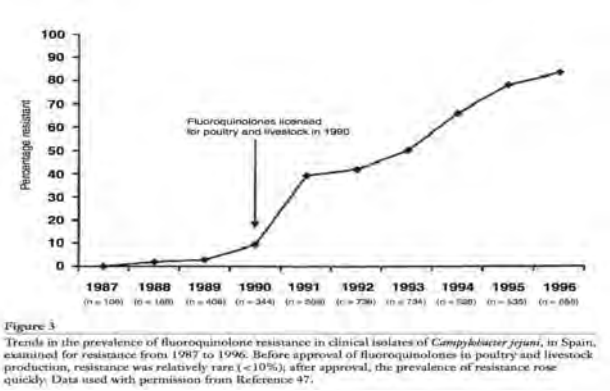
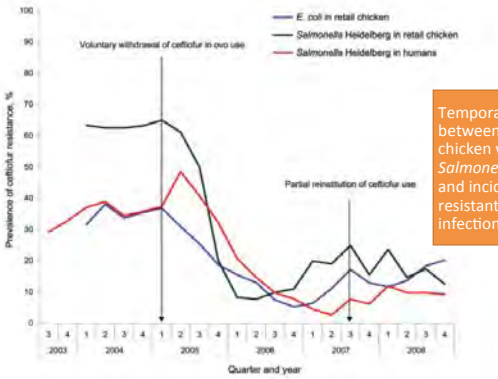


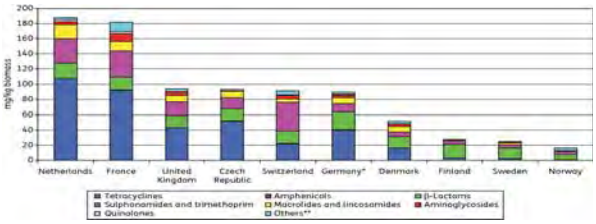
Figure 3
Trends in the prevalence of fluoroquinolone resistance in clinical isolates of *Campylobacter jejuni*, in Spain, examined for resistance from 1987 to 1996. Before approval of fluoroquinolones in poultry and livestock production, resistance was relatively rare (<10%); after approval, the prevalence of resistance rose quickly. Data used with permission from Reference 47.



Temporal association between contamination of retail chicken with ceftiofur-resistant *Salmonella* Heidelberg strains and incidence of ceftiofur resistant *Salmonella* Heidelberg infection in humans

Dutil et al, EID, 2010

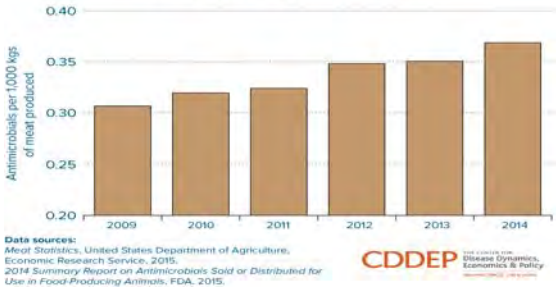
Amounts, in mg, of veterinary antibacterial agents sold in 2007 per kg biomass of pig meat, poultry meat and cattle meat produced plus estimated live weight of dairy cattle. *2005 data. **The substances included vary from country to country.



Grave K et al. J. Antimicrob. Chemother. 2010;65:2037-2040

Journal of Antimicrobial Chemotherapy

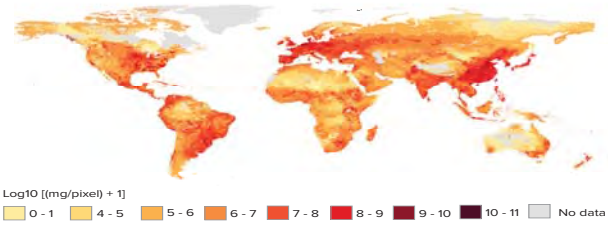
Antimicrobial use per unit of meat has increased every year from 2009 to 2014 in the US.



Data sources:
Meat Statistics, United States Department of Agriculture, Economic Research Service, 2015.
2014 Summary Report on Antimicrobials Sold or Distributed for Use in Food-Producing Animals, FDA, 2015.

CDDEP Center for Disease Dynamics, Economics & Policy

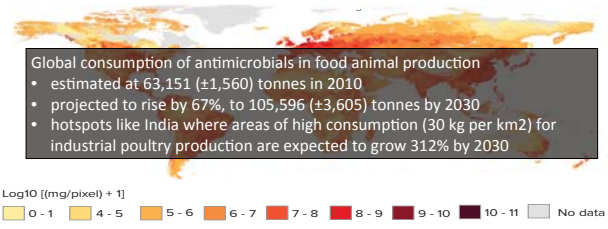
Global antibiotic consumption in livestock (mg per 10 km² pixels) 2010



Van Boeckel et al., PNAS, 2015

CDDEP Center for Disease Dynamics, Economics & Policy

Global antibiotic consumption in livestock (mg per 10 km² pixels) 2010

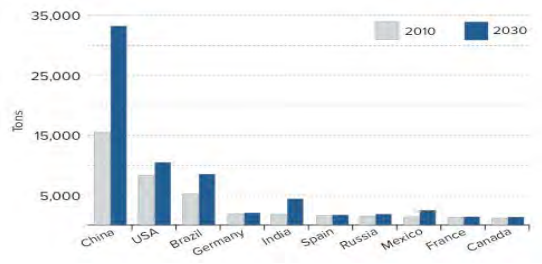


Global consumption of antimicrobials in food animal production
- estimated at 63,151 (±1,560) tonnes in 2010
- projected to rise by 67%, to 105,596 (±3,605) tonnes by 2030
- hotspots like India where areas of high consumption (30 kg per km2) for industrial poultry production are expected to grow 312% by 2030

Van Boeckel et al., PNAS, 2015

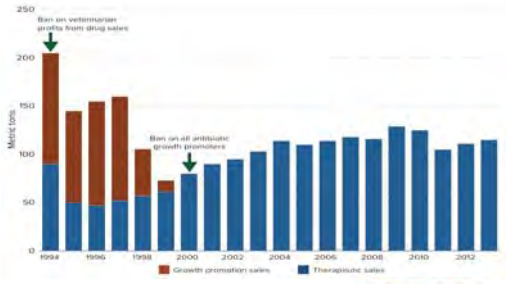
CDDEP Center for Disease Dynamics, Economics & Policy

Antibiotic consumption in livestock, top ten countries 2010–2030 (projected for 2030)

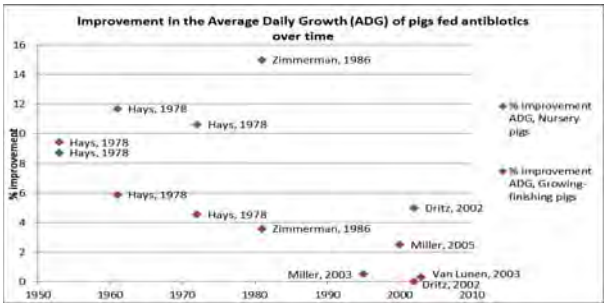


Van Boeckel et al., PNAS, 2015

Sales of active ingredients of antibiotics for food-producing animals in Denmark



DANMAP 2013 (adapted)

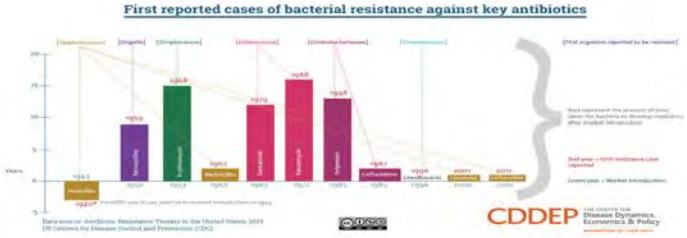


Laxminarayan et al, OECD Report, 2015

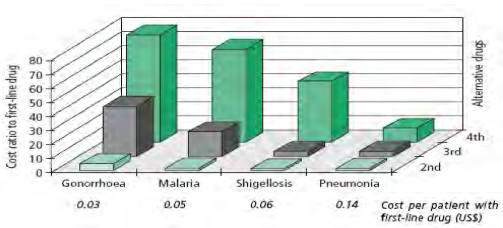
Productivity reductions and costs per produced pig incurred by removing AGPs

Laxminarayan et al, OECD Report, 2015

IV. Is finding new antibiotics the answer?

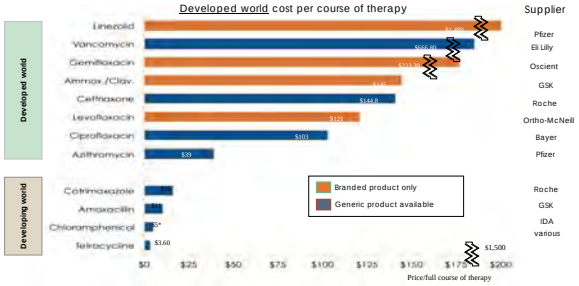


Loss of first line drugs increases drug costs



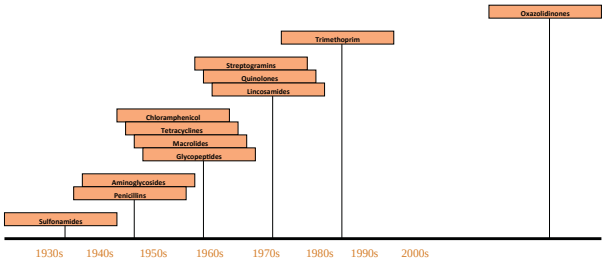
Source: WHO Policy Perspective 2005, adapted from WHO Model Formulary, WHO Clinical Guidelines and Management Sciences for Health's 2004 International Drug Price Indicator Guide (slide courtesy: David Heymann)

The rich pay with their wallets, the poor with their lives



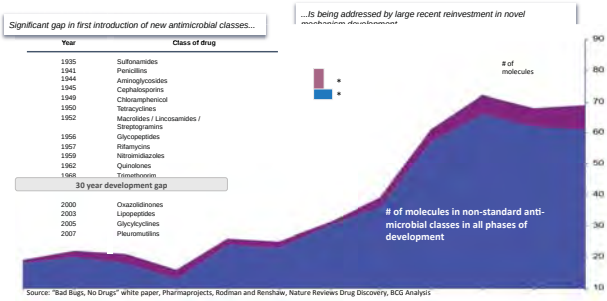
Notes: *Chloramphenicol is not available in developed world– price is therefore estimated. †Ceftriaxone and ciprofloxacin may be available in some tertiary settings in developing world.
Source: The Medical Letter (2006), Disease control priorities in developing countries, Lancet (2006), Expert interviews.

Discovery of new classes of antibiotics



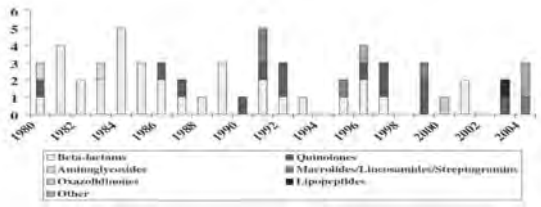
Pipeline of new anti-microbial drugs growing after a long lag

But prices are likely to be high

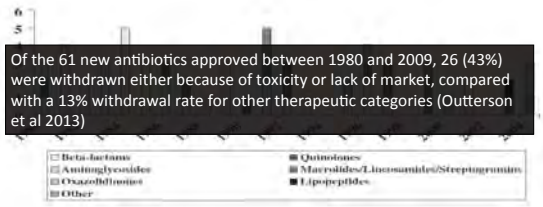


Source: "Bad Bugs, No Drugs" white paper, Pharmaprojects, Rodman and Renshaw, Nature Reviews Drug Discovery, BCG Analysis

Trends in development of new antibiotics



Trends in development of new antibiotics



Of the 61 new antibiotics approved between 1980 and 2009, 26 (43%) were withdrawn either because of toxicity or lack of market, compared with a 13% withdrawal rate for other therapeutic categories (Outterson et al 2013)

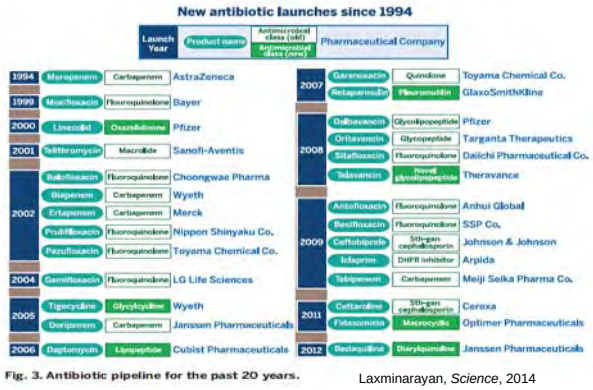
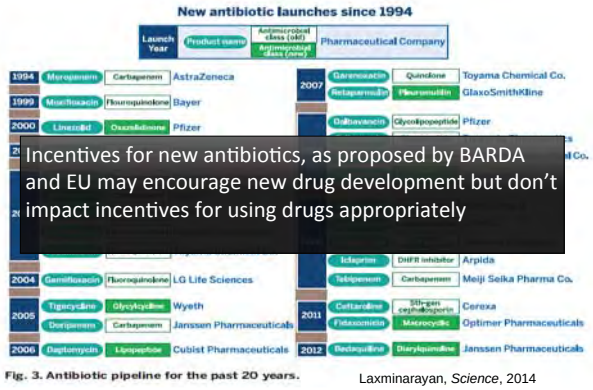


Fig. 3. Antibiotic pipeline for the past 20 years.

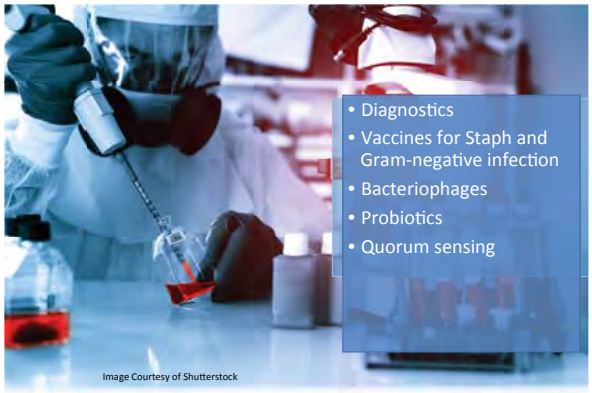
Laxminarayan, *Science*, 2014

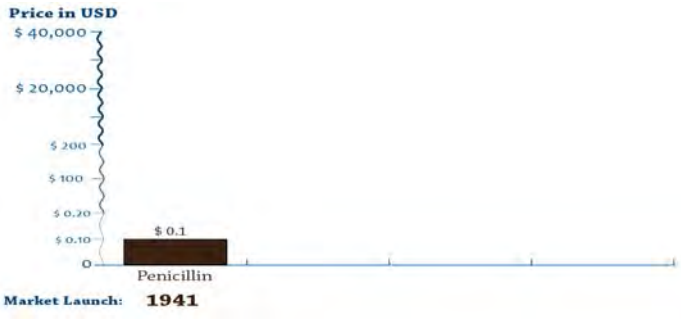


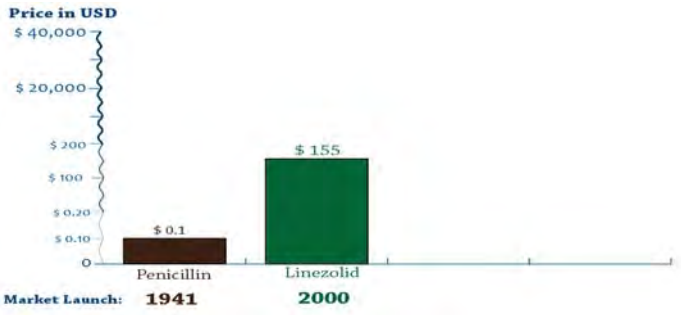
Incentives for new antibiotics, as proposed by BARDA and EU may encourage new drug development but don't impact incentives for using drugs appropriately

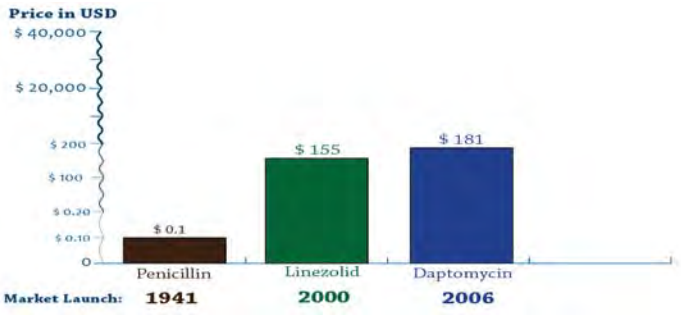
Fig. 3. Antibiotic pipeline for the past 20 years.

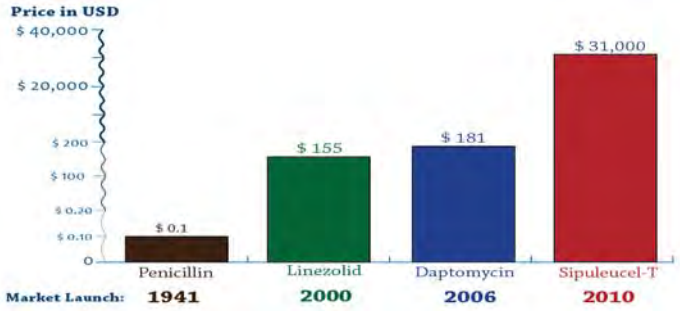
Laxminarayan, *Science*, 2014

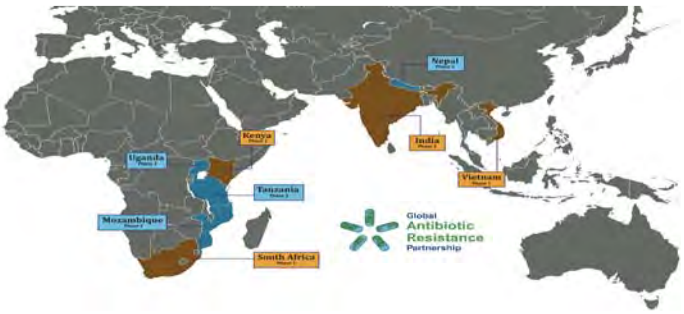












United Nations A/71/L.2

General Assembly

Distr.: Limited
22 September 2016

Original: English

Seventy-first session
Agenda item 127
Global health and foreign policy

Draft resolution submitted by the President of the General Assembly

Political Declaration of the high-level meeting of the General Assembly on antimicrobial resistance

BLADE OF GRASS IS RESPONSIBLE FOR LOSS OF FOOT

C. W. Jones, athletic director of the Athens Y. M. C. A. yesterday suffered the loss of his right foot, the member having been amputated just above the ankle.

Mr. Jones, it seems, recently was exercising on a plot of grass, dew on a blade of grass cutting him slightly just under the little toe. The cut did not heal as quickly as it should have and medical attention was called, but to no avail. Blood poisoning had set in, and it was imperative that the foot be amputated to prevent the poison spreading further.

Weekly Banner, 18 July 1894, p. 2, col. 2.
©Athens-Clarke County Heritage Room, 2014.

Image Courtesy of Shutterstock

Resistancemap.org

Slides are downloadable @ www.cddep.org

Thank you

Antimicrobial Stewardship: Health Canada's Efforts to Strengthen Canada's Regulatory Framework for Veterinary Antimicrobials

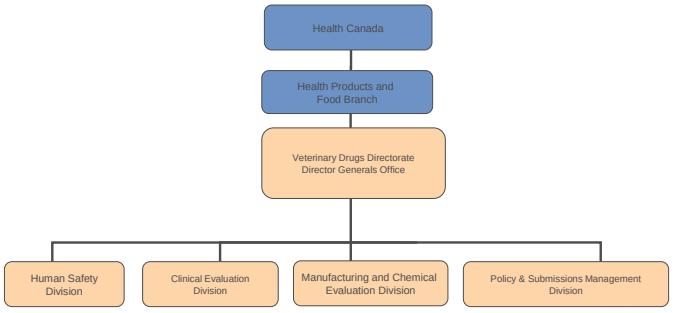
Presented to the Animal Nutrition Conference of Canada
May 10 - 11, 2017

YOUR HEALTH AND SAFETY ... OUR PRIORITY

Outline

- Veterinary Drug Regulation in Canada
- AMR as a cross-cutting Public Health Issue
- Overview and update on Health Canada led-AMR initiatives to enhance Antimicrobial Stewardship
- Next steps

Organizational Chart



Federal Regulatory Authorities

- **VDD Mandate**: To protect human and animal health and the safety of Canada's food supply, the Veterinary Drugs Directorate (VDD) evaluates and monitors the safety, quality and effectiveness, sets standards, and promotes the prudent use of veterinary drugs administered to food-producing and companion animals
- *Food and Drugs Act and Regulations*
  - Sale and advertising of food, drugs, natural health products, and medical devices in Canada
- *Feeds Act & Regulations* (under the Canadian Food Inspection Agency)
  - Regulation of Drugs in Livestock Feed
  - *Compendium of Medicating Ingredients Brochures* stipulate the drugs to be included in feeds
    http://www.inspection.gc.ca/english/anima/feebet/mib/mibe.shtml
- **What we do not have authority over**
  - Practice of medicine: compounding, drug use, extra-label drug use

VDD's Core Activities

Regulatory oversight throughout the lifecycle of veterinary drugs:

- **Drug Submissions Review:**
  - **Pre-Market** – review of veterinary drug submissions from industry and establishment of limits for veterinary drugs in foods – safety, efficacy, quality
  - **Post-Market** – pharmacovigilance, advice on enforcement and compliance of drugs as well as food (support role to Inspectorate & CFIA)
- **Priorities –** Antimicrobial Resistance, Veterinary Health Products, Drug vs Feed, Drug Compatibilities in-feed, Minor Use Minor Species, International Regulatory Collaboration

Antimicrobial Resistance

Antimicrobials are essential for the treatment, control and prevention of bacterial infections in humans and animals…

- Microbes can change in ways that reduce or eliminate the effectiveness of antimicrobial action (i.e. the treatment of infections).
- Antimicrobial resistance (AMR) = reduced or eliminated effectiveness of antimicrobials.
- Antimicrobial drugs are used across multiple sectors which indicates complex contributing factors
  - Health care
  - Agriculture
  - Environment
  - Consumer products

…but the inappropriate use of antimicrobials in all sectors is leading to increases in the emergence and spread of AMR

Why is the world worried about AMR?

- By 2050, annual deaths due to AMR could reach 10 million worldwide, overtaking deaths due to diabetes and cancer combined, and is expected to cost the global economy $100 trillion USD[1]
- Every year, over 20,000 hospital patients in Canada develop infections that are resistant to antimicrobial drugs, resulting in over $250M in direct medical costs[2]
- Resistance can emerge from any country and spread: travel, medical tourism, the shipment of food and animals, environmental contamination and the food chain are vehicles for the spread of AMR
  - MCR-1, a gene that increases resistance to important antibiotics, emerged in China and has since spread to countries around the world, including Canada and the United States

1. O'Neill, *Review on Antimicrobial Resistance May, 2016*
2. *CIHR Statement on World Antibiotic Awareness Week 2016*

A global response is underway

- On September 21, 2016 the President of the United Nations General Assembly convened a High Level Meeting (HLM) of Member States on AMR
  - AMR was recognized as a challenge to health, food security, and development
  - The World Health Organization Global Action Plan on AMR (WHO GAP) was also recognized as the blueprint for action
- Global Action Plan on AMR endorsed by Member States at the World Health Assembly, including Canada (May 2015)
  - Requires countries to have national plans in place by May 2017
  - United States, United Kingdom, European Union and others have already developed and funded national AMR strategies, with leadership at the highest level
- G7 and G20 have identified AMR as a priority at the Leaders level, and by Ministers of Health, Agriculture and Science
- The Global Health Security Agenda has also identified AMR as a priority
  - Canada is one of the countries that co-leads the Action Package on AMR; will Chair in 2017
- Multi-lateral organizations and NGOs are contributing to or supporting work on AMR (e.g., World Organisation for Animal Health (OIE), Food and Agriculture Organization (FAO), Wellcome Trust, Codex Alimentarius Commission)

Government of Canada response to AMR

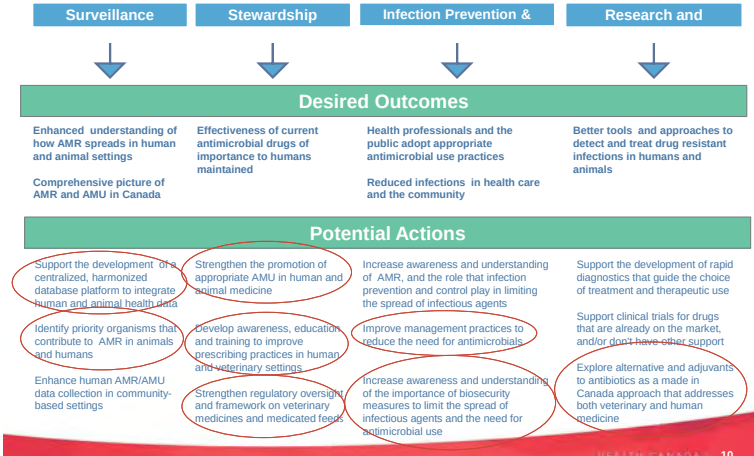
- The Government of Canada is addressing AMR through a multi-sectoral "One Health" Approach:

October 2014: Release of *Antimicrobial Resistance and Use in Canada: A Federal Framework for Action* outlining strategic objectives in the areas of:
- **Surveillance** activities that enhance and integrate human health, animal health and agri-food surveillance systems;
- **Stewardship** activities, including increased participation in Antibiotic Awareness Week and enhanced regulatory oversight for antimicrobials;
- **Innovation** through federally supported research, international collaboration and public/private partnership

March 2015: Release of Federal *Action Plan on Antimicrobial Resistance and Use in Canada: Building on the Federal Framework for Action* which builds on the *Framework* strategic areas of focus by identifying concrete steps that the Government of Canada will undertake

Work underway on developing a Pan-Canadian Framework on AMR

Draft Pan-Canadian Framework on AMR



Managing AMR in Veterinary Drugs Context

- **AMR risk from animals** is one part of a multi-faceted problem
  - In Canada, an estimated 70 % of all medically important antimicrobial drugs are sold for use in food-producing animals
  - Shared jurisdiction (F/P/T) over sale and use of veterinary medicines
  - Multiple stakeholders, including federal, P/T and municipal governments, industry and stakeholders all have a role to play to manage AMR risk

Role of Health Canada as a Federal Regulator in Antimicrobial Stewardship:

- Ensuring safe and effective drug products are available on the market
- Proper oversight over importation and sale of safe and effective drugs
- Promoting prudent use of such products
- Work with other partners to support their stewardship activities (P/Ts, animal nutritionists, veterinarians etc.)

Current Activities to address AMR

- We are making important Regulatory and Policy changes to strengthen prudent use of Medically important antimicrobials (MIAs) in livestock production

Snapshot – Veterinary Drugs AMR Initiatives Underway

These regulatory and policy initiatives are interconnected and mutually supportive:

1) Increasing oversight on importation of veterinary drugs (Own Use Importation)

- *new regulatory proposal*

2) Increasing oversight on importation and quality of active pharmaceutical ingredients (APIs)

- *new regulatory proposal*

3) Mandatory reporting of sales volume from manufacturers and importers to support antimicrobial use surveillance

- *new regulatory proposal*

4) Facilitating access to low risk veterinary health products (VHPs), as additional tools for the maintenance of animal health and welfare

- *new regulatory proposal and existing policy tools*

5) Removing growth promotion claims from medically-important antimicrobials

- *policy under existing regulatory tools*

6) Increasing veterinary oversight over all MIAs (Prescription status switch)

- *policy under existing regulatory tools*

1) Oversight on Importation of vet drugs (OUI)

Current Situation

– Veterinary drugs, including over the counter antimicrobials, can be imported to Canada for own use purposes with limited regulatory oversight. In this context, own use importation refers to importation by an individual for use on animal(s) under their care or guardianship, and not for further sale.

Regulatory proposal

– Prohibits importation of unapproved drugs for own use, with an exemption for specified drug products that do not represent an unacceptable risk to food safety and public health

– Exempted product list to be *Incorporated by Reference* and established based on specified criteria established by Health Canada

– No MIAs or Pr drugs will be allowed to be imported for own use purposes for use in food-producing animals

2) Oversight on Importation and Quality of APIs

Current Situation

– There is limited oversight on the importation of antimicrobials as APIs for veterinary use.

– Currently, manufacturers, importers and compounders of APIs for veterinary use are not required to have an Establishment Licence (EL) or to follow Good Manufacturing Practices (GMPs).

Regulatory proposal

– Expand existing regulatory requirements of GMPs for APIs used in human drugs to all veterinary APIs

– Restrictions on who can import MIAs (e.g. Importation of MIA APIs by food animal producers for their direct use in food animals will not be allowed)

– Require an EL for individuals seeking to import APIs for MIA drugs

3) Mandatory Reporting of antimicrobial sales volume

Current Situation

– No Regulatory authority to collect sales volume for drugs

Regulatory proposal

– Require manufacturers or importers of veterinary drugs in dosage form that contain an API for medically important antimicrobial to provide on an annual basis, a report identifying for each drug the total quantity sold and an estimate of the quantity sold for each intended animal species; and

– Require persons, including pharmacists and practitioners, that import and compound and sell an API for medically important antimicrobial drugs (List A) for veterinary use to provide on an annual basis the same report

Data gathered will support the surveillance pillar of the Federal Action Plan…

4) New Pathway for Veterinary Health Products (VHPs)

Current Situation

– No regulatory provisions for sale of low risk veterinary health products

Regulatory proposal

– Creating a risk-based regulatory pathway to allow importation and sale of low risk veterinary health products for use in animals, including food animals

– The proposal builds on the successes and lessons learned from the "Interim Notification Pilot Program (INPP)" for companion animal drugs, and would need continued support from Producer groups and on Farm Food Safety Programs

5) Removal of Growth Promotion Claims

– Phase out non-prudent uses of MIAs in animals for long-term non-therapeutic purposes i.e. growth promotion and weight gain

– No growth promotion claims approved for new MIAs post-2004

– There is lack of modern data to show that these products are still effective at the approved dosage (approved several decades ago)

– Positive responses from manufacturers of all implicated products; overall support from food animal producers, veterinary professionals and other stakeholders

– About 64 products are implicated

– Minimizing impact on availability of treatment options

6) Increasing Veterinary Oversight of all MIAs (Pr)

- Moving all existing over the counter MIAs to the Prescription Drug List (Pr status);
- All in-feed MIAs to be included in CMIB; and require a Prescription (Pr) prior to sale for on-label products
- About 300 products implicated in all dosage forms (with about 75 in-feed MIAs)

Update Notice to Stakeholders posted February 13, 2017:

- **http://www.hc-sc.gc.ca/dhp-mps/vet/antimicrob/amr-notice-ram-avis-2017-eng.php**

6) Increasing Veterinary Oversight over all MIAs (contd..)

Implicated MIAs for switch from OTC to Pr status

- Apramycin
- Bacitracin
- Erythromycin
- Lincomycin
- Neomycin
- Penicillin G
- Spectinomycin
- Streptomycin/Dihydrostreptomycin
- Sulphonamides
- Tetracycline/Chlortetracycline/Oxytetracycline
- Tilmicosin
- Tiamulin
- Tylosin/Tylvalosin
- Virginiamycin
- Or their salts or derivatives

Who can sell Prescription Drugs?

The responsibility for the sale, dispensing and distribution of prescription drugs is shared between federal and provincial/territorial authorities

Federal level:

- Categories of individuals who are allowed to sell a prescription drug are specified in the *Food and Drug Regulations* including record keeping requirements

Provincial/Territorial level:

- Provincial/Territorial rules may specify individuals who are entitled under the laws of a province/territory to dispense prescription drug and to sell it in that province

Path Forward for In-feed Medications containing MIAs

- **A veterinary prescription will be required prior to sale when an MIA drug is mixed in a livestock feeds.**
- All the approved in-feed drugs (including OTC and Pr) to be included in the Canadian Medicating Ingredients Brochure (CMIB).
- There will be no restriction on manufacturing (floor stocking) of such MIA-medicated feeds if manufactured pursuant to Health Canada approvals (i.e. as per CMIB).
- Restrictions remain if manufacturing a medicated feed in a manner deviating from Health Canada's approvals and Veterinary prescriptions will continue to be required prior to manufacturing (i.e. no floor stocking).

Update & Next Steps

- Formal 75 day Canada Gazette, Part I consultation – July 2 to September 14, 2016
- Anticipating Canada Gazette, Part II publication of final regulations in 2017
- Work underway on implementation details and guidance documents, incorporating feedback received
- Implementation considerations for the proposed regulations as well as the policy initiatives

Health Canada Santé Canada

Canada

VDD's AMR initiatives roll up to just one piece of this complex puzzle… Need Continued Collaboration and Support from key Stakeholders like you!

YOUR HEALTH AND SAFETY ... OUR PRIORITY

Health Canada Santé Canada

Canada

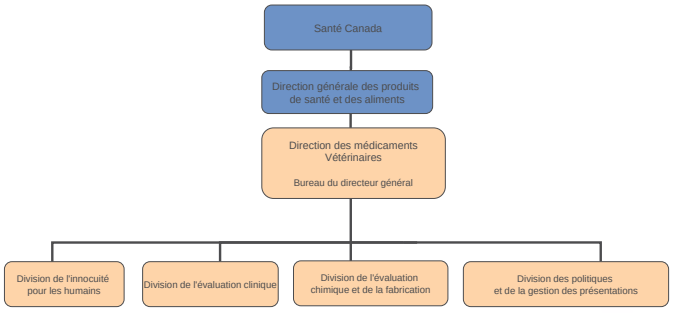
Gestion des antimicrobiens: efforts de Santé Canada visant à renforcer le cadre réglementaire pour les produits vétérinaires antimicrobiens

Présentation au Colloque de nutrition animale du Canada
10 – 11 mai 2017

Aperçu

- Réglementation des médicaments vétérinaires au Canada
- RAM est un problème de santé publique à facettes multiples
- Aperçu et mise à jour des initiatives de Santé Canada sur la RAM pour en améliorer l'intendance
- Prochaines étapes

Organigramme



Mandat et autorités réglementaires de la DMV

- **Mandat de la DMV**: Pour veiller à la bonne santé des humains et des animaux, ainsi qu'à la sécurité de l'approvisionnement alimentaire au Canada, la Direction des médicaments vétérinaires (DMV) évalue et contrôle la sécurité, la qualité et l'efficacité des médicaments vétérinaires et établit des normes pour une utilisation prudente et responsable des médicaments vétérinaires administrés aux animaux producteurs de denrées alimentaires, ainsi qu'aux animaux de compagnie.

- *Loi et Règlement sur les aliments et drogues*
  - Vente et publicité d'aliments, de médicaments, de produits de santé naturels et de matériel médical au Canada

- *Loi et Règlement sur les aliments du bétail* (Agence canadienne d'inspection des aliments)
  - Réglementation de l'ajout de médicaments aux aliments pour le bétail
  - Les médicaments dont l'ajout aux aliments du bétail est autorisé sont énumérés dans le *Recueil des notices sur les substances médicatrices* http://www.inspection.gc.ca/animaux/aliments-du-betail/substances-medicatrices/fra/1300212600464/1320602461227

- **Domaines hors de notre zone d'intervention**
  - Pratique de la médecine : préparation de médicaments, utilisation de médicaments et utilisation de médicaments « hors étiquette ».

Activités principales de la DMV

Surveillance réglementaire tout au long du cycle de vie d'un produit vétérinaire :

- **Examen des présentations de médicaments**
  - **Avant la mise en marché** – examen des présentations de médicaments provenant de l'industrie et établissement de limites maximales de résidus de médicaments vétérinaires dans les aliments
  - **Après la mise en marché** – pharmacovigilance, conformité et application de la loi (rôle de soutien à l'Inspectorat et l'ACIA)

- **Priorités –** Résistance aux antimicrobiens, Produits de Santé Vétérinaires, Drogue contre Aliment, Compatibilité des médicaments dans les aliments, Usage limité pour espèces mineures, Collaboration réglementaire internationale

Résistance aux antimicrobiens

Les antimicrobiens sont essentiels pour le traitement, controle et prévention des infections bactériennes chez les humains et les animaux…

- Les microbes peuvent évoluer de manière à réduire ou éliminer l'efficacité de l'action antimicrobienne (par exemple le traitement des infections).

- Résistance aux antimicrobiens (RAM) = efficacité réduite ou éliminée des antimicrobiens.

- Les médicaments antimicrobiens sont utilisés à travers plusieurs secteurs ce qui indique des facteurs contributifs complexes
  - » Santé
  - » Agriculture
  - » Environnement
  - » Produits de consommation

… mais l'utilisation inappropriée des antimicrobiens dans tous les secteurs amène à des augmentations dans l'émergence et la propagation de la résistance aux antimicrobiens

Pourquoi est ce que le monde s'inquiète de la RAM?

- D'ici 2050, les morts annuelles dues à la RAM pourraient atteindre 10 millions à travers le monde, chiffres plus élevés que ceux de la mortalité due au diabète et cancer, et pourrait coûter $100 trilliards (USD) à l'économie mondiale [1]
- Chaque année, plus de 20,000 patients dans les hopitaux au Canada développent des infections qui résistent aux médicaments antimicrobiens, ce qui résulte à plus de $250M de coûts médicaux directs [2]
- La résistance peut émerger de tout pays et se propager: voyages, tourisme médical, l'envoi d'aliments et d'animaux, la contamination environnementale et la chaine alimentaire sont autant de véhicules pour la propagation de la RAM
  - MCR-1, un gène qui augmente la résistance aux antibiotiques importants, a emergé en Chine et depuis s'est propagé dans les pays à travers le monde, dont le Canada et les États-Unis

1. O'Neill, *Review on Antimicrobial Resistance May 2016*
2. *Déclaration de l'Institut de recherche en santé du Canada, à la Semaine mondiale pour un bon usage des antibiotiques 2016*

Une réponse mondiale est en voie

- Le 21 septembre 2016, le président de l'Assemblée générale des Nations Unies a convoqué une réunion de haut niveau des États membres sur la RAM
  - La RAM a été reconnue comme un défi à la santé, à la sécurité alimentaire, et au développement
  - Le Plan d'Action mondial de l'Organisation mondiale de la Santé sur la résistance aux antimicrobiens a aussi été reconnu comme un modèle pour action
- Plan d'Action mondial sur la RAM a reçu l'appui des États membres à l'Assemblée mondiale de la Santé, dont le Canada (Mai 2015)
  - Exige des pays d'avoir des plans domestiques en place d'ici mai 2017
  - Les États-Unis, le Royaume-Uni, l'Union Européenne et autres ont déjà développé et financé des stratégies nationales sur la RAM, avec un leadership au plus haut niveau
- Les G7 et G20 ont identifié la RAM comme une priorité au niveau de leurs leaders, et aussi par les ministres de la santé, de l'agriculture et de la science
- Le Programme de sécurité sanitaire mondiale a aussi identifié la RAM comme une priorité
  - Canada est un des pays qui co-mène le plan d'action sur la RAM, et présidera en 2017
- Des organisations multilatérales et des ONG contribuent ou appuient le travail sur la RAM (par exemple l'Organisation mondiale de la santé animale (OIE), l'Organisation des Nations Unies pour l'alimentation et l'agriculture (FAO), le Wellcome Trust, la Commission du Codex Alimentarius)

Réponse du gouvernement du Canada à la RAM

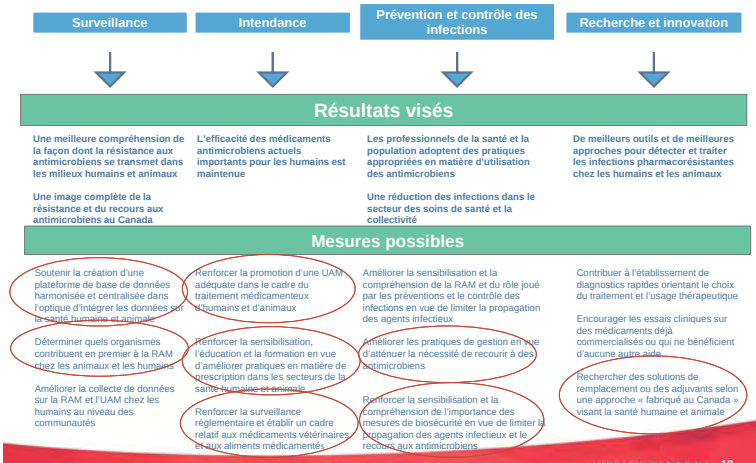
- Le gouvernement du Canada fait face à la RAM à travers une approche à multi-facettes "Une Santé":

Octobre 2014: Publication du *Résistance et recours aux antimicrobiens au Canada : cadre d'action fédéral* qui décrit les objectifs stratégiques dans les domaines suivants :
- Activités de **Surveillance** qui améliorent et intégrent les systèmes de surveillance de santé humaine, de santé animale, et agro-alimentaire;
- Activités d'**Intendance**, notamment une participation accrue à la Semaine pour un bon usage des antibiotiques, et une surveillance réglementaire améliorée des antimicrobiens;
- **Innovation** à travers la recherche supportée par le fédéral, la collaboration internationale, et les partenariats publiques/privés

Mars 2015: Publication du *Plan d'action fédéral sur la résistance et le recours aux antimicrobiens au Canada : Prolongement du cadre d'action fédéral* qui repose sur les domaines stratégiques du *Cadre* en définissant les étapes concrètes qui seront entreprises par le gouvernement du Canada

Travail en cours pour élaborer un cadre pancanadien visant la résistance aux antimicrobiens

Ébauche du cadre pancanadien sur la RAM



Gestion de la RAM dans le contexte des médicaments vétérinaires

- **Le risque de RAM chez les animaux** est l'un des éléments d'un problème à multiples facettes
  - On estime qu'au Canada, 70 % de la totalité des antimicrobiens importants sur le plan médical sont utilisés chez des animaux destinés à la consommation
  - La réglementation de la vente et de l'utilisation des médicaments vétérinaires sont de compétence partagée (F/P/T)
  - Une multitude d'intervenants, dont les gouvernements fédéral, P/T et municipaux, ainsi que l'industrie ont tous un rôle à jouer dans la gestion du risque de la RAM

Rôle de Santé Canada comme régulateur fédéral dans la gestion des antimicrobiens :

- S'assurer de l'innocuité et de l'efficacité des médicaments disponibles sur le marché
- Surveillance appropriée de l'importation et de la vente de médicaments inoffensifs et efficaces
- Promouvoir l'utilisation de tels produits
- Travailler avec d'autres partenaires pour appuyer les activités d'intendance (P/Ts, nutrionistes pour animaux, vétérinaires etc.)

Activités en cours pour faire face à la RAM

- Nous faisons des changements réglementaires et politiques importants pour renforcer l'utilisation prudente des antimicrobiens importants sur le plan médical (AIM) dans la production d'animaux d'élevage

Aperçu – Initiatives liées à la RAM en cours

Ces initiatives réglementaires et politiques sont interreliées et complémentaires :

1) Accroître la surveillance de l'importation des médicaments vétérinaires (importation pour usage personnel)

- *Nouveau projet de règlement*

2) Accroître la surveillance de l'importation et la qualité des ingrédients pharmaceutiques actifs (IPA)

- *Nouveau projet de règlement*

3) Déclaration obligatoire des volumes de ventes des fabricants et importateurs pour appuyer la surveillance de l'utilisation des agents antimicrobiens

- *Nouveau projet de règlement*

4) Faciliter l'accès à des produits de santé vétérinaires à faible risque, à titre d'outils supplémentaires pour favoriser la santé et le bien-être animal

- *Nouveau projet de règlement et outils de politique existants*

5) Éliminer les allégations de stimulation de la croissance des médicaments antimicrobiens importants sur le plan médical

- *Approche en matière de politique*

6) Accroître la surveillance vétérinaire de tous les médicaments antimicrobiens importants sur le plan médical (passage à la vente sur ordonnance)

- *Outils de politique et outils de réglementation existants*

1) Surveillance de l'importation des médicaments vétérinaires (importation pour usage personnel [IUP]))

Situation actuelle

- Les médicaments vétérinaires, y compris les antimicrobiens en vente libre, peuvent être importés au Canada pour un usage personnel en étant soumis à une surveillance réglementaire limitée. Dans ce contexte, l'importation pour usage personnel fait référence à l'importation par une personne en vue d'utiliser le médicament sur un animal dont elle a la charge ou la garde, non pas en vue de le revendre.

Projet de règlement

- Interdire l'importation des médicaments non approuvés pour usage personnel, exception faite de certains produits pharmaceutiques qui ne représentent pas un risque inacceptable pour la salubrité alimentaire et la sécurité publique
- Liste des produits exemptés *à incorporer par voie de référence* et établie en fonction de critères précis fixés par Santé Canada
- On ne permettra pas l'IUP de médicaments antimicrobiens importants sur le plan médical chez les animaux destinés à l'alimentation

2) Surveillance de l'importation et de la qualité des IPA

Situation actuelle

- L'importation des antimicrobiens comme IPA destinés à un usage vétérinaire fait l'objet d'une surveillance limitée.
- À l'heure actuelle, les fabricants, importateurs et transformateurs d'IPA destinés à un usage vétérinaire ne sont pas tenus de détenir une licence d'établissement (LE) ni de suivre de bonnes pratiques de fabrication (BPF).

Projet de règlement

- Étendre à l'ensemble des IPA des médicaments vétérinaires la portée des exigences réglementaires actuelles liées aux BPF pour les IPA utilisés dans les médicaments destinés à l'usage humain.
- Imposer des restrictions concernant les personnes qui peuvent importer des produits antimicrobiens d'importance médicale (p. ex., l'importation d'IPA de produits antimicrobiens d'importance médicale par les producteurs d'animaux destinés à l'alimentation ne sera pas permise).
- Exiger une LE pour les vétérinaires qui cherchent à importer des IPA pour des médicaments AIM.

3) Déclaration obligatoire des volumes de ventes des antimicrobiens

Situation actuelle

- Il n'existe aucune autorité réglementaire chargée de consigner les volumes de vente des médicaments.

Projet de règlement

- Les fabricants ou importateurs de médicaments vétérinaires sous forme posologique finale qui renferment des IPA pour des produits antimicrobiens importants sur le plan médical devront présenter chaque année un rapport faisant état, pour chaque médicament, de la quantité totale vendue et une estimation de la quantité vendue pour chaque espèce animale visée ; et
- Les personnes, y compris les pharmaciens et les praticiens, qui importent et fabriquent un IPA pour des produits antimicrobiens importants sur le plan médical pour usage vétérinaire (liste A), doivent présenter chaque année le même rapport.

Les données réunies appuieront le volet de surveillance du Plan d'action fédéral.

4) Nouvelle voie réglementaire pour les produits de santé vétérinaires

Situation actuelle

- Il n'existe aucune disposition relative à la vente de produits de santé vétérinaire à faible risque.

Projet de règlement

- Création d'une nouvelle voie réglementaire fondée sur les risques pour permettre l'importation et la vente de produits de santé vétérinaires à faible risque destinés à être utilisés chez les animaux, dont ceux qui sont destinés à l'alimentation.
- La proposition s'appuie sur les réussites et leçons dégagées du programme pilote de déclaration provisoire (PPDP) pour les médicaments destinés aux animaux de compagnie et elle devra recevoir l'appui continu des groupes de producteurs et des divers programmes de salubrité des aliments à la ferme.

5) Retrait des allégations relatives à la stimulation de la croissance figurant sur les étiquettes

- Élimination progressive chez les animaux des utilisations non prudentes des AIM à des fins non thérapeutiques de longue durée, c.-à-d. la stimulation de croissance / gain de poids
- Aucune allégation de stimulation de croissance n'a été approuvée pour de nouveaux AIM après 2004
- Il y a peu de données récentes démontrant que ces produits sont toujours efficaces aux doses approuvées (et datant parfois de plusieurs décennies)
- Appui des fabricants des produits concernés, et appui d'ensemble de la part des producteurs d'animaux destinés à la consommation, des professionnels vétérinaires et autres intervenants
- Environ 64 produits sont touchés
- Impact limité sur la disponibilité des options de traitement

6) Renforcer la surveillance vétérinaire sur tous les AIM utilisés (Pr)

- Inclure tous les AIM en vente libre à la Liste des drogues sur ordonnance (LDO) existante (statut Pr)
- Inclusion de tous les AIM ajoutés aux aliments des animaux dans le RNSM et exigence d'une prescription avant la vente pour les produits sur l'étiquette
- Environ 300 produits touchés, tous modes d'administration compris (incluant près de 75 AIM ajoutés aux aliments des animaux)

Nouvel avis aux intervenants le 13 février 2017 :

- **http://www.hc-sc.gc.ca/dhp-mps/vet/antimicrob/amr-notice-ram-avis-2017-fra.php**

6) Renforcer la surveillance vétérinaire sur tous les AIM utilisés (suite...)

AIM qui passeront de vente libre à sur ordonnance

- Apramycine
- Bacitracine
- Erythromycine
- Lincomycine
- Néomycine
- Pénicilline G
- Spectinomycine
- Streptomycine/Dihydrostreptomycine
- Sulfonamides
- Tétracycline/Chlortétracycline/Oxytétracycline
- Tilmicosine
- Tiamuline
- Tylosine/Tylvalosine
- Virginiamycine
- Ou leurs sels et dérivés

Qui peut vendre des drogues sur ordonnance?

La responsabilité pour la vente, la livraison et la distribution de drogues sur ordonnance est partagée entre les autorités fédérale et provinciales/territoriales

Niveau fédéral :

- Les catégories d'individus qui sont autorisés à ventre une drogue sur orodnnance sont spécifiés dans le *Règlement sur les aliments et drogues,*et notamment les exigences de tenus de registres

Niveau provincial/territorial :

- Les règles provinciales/territoriales peuvent indiquer quels individus sont autorisés par les lois de la province/territoire de délivrer et de vendre une drogue sur ordonnance

La voie à suivre pour les médicaments dans les aliments contenant des AIM

- **Une ordonnance vétérinaire sera exigée avant la vente lorsqu'un AIM est mélangé aux aliments du bétail.**
- Inclure toutes les drogues (en vente libre et sur ordonnance) administrées avec les aliments dans le Recueil des notices sur les substances médicatrices (RNSM).
- Aucune restriction ne sera imposée à la fabrication (entreposage sur le plancher) de tels aliments médicamentés avec AIM s'ils sont fabriqués en conformité avec les approbations de Santé Canada (c.-à-d. en vertu du RNSM).
- Des restrictions demeureront si la fabrication d'un aliment médicamenté n'est pas réalisée en conformité avec les approbations de Santé Canada et une ordonnance vétérinaire continuera d'être exigée avant la fabrication (c.-à-d. pas d'entreposage sur le plancher).

Le point et la suite des choses

- Consultation officielle de 75 jours, *Gazette du Canada*, partie I, du 2 juillet au 14 septembre 2016
- Nous anticipons une publication du règlement final dans la *Gazette du Canada*, Partie II en 2017
- Des travaux sont en cours sur les détails et l'orientation en matière de mise en œuvre, en tenant compte des commentaires reçus
- Des considérations de mise en œuvre sont prises en compte pour les modifications réglementaires proposées ainsi que les initiatives de politique

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The End of Antimicrobials: Biological Reality or Consumer Choice?

La fin des antimicrobiens : réalité biologique ou choix des consommateurs?

Tim A. McAllister
Principal Research Scientist
Agriculture and Agri-Food Canada
Lethbridge Research Centre
Lethbridge, AB, T1J 4B1
tim.mcallister@agr.gc.ca

Abstract

Demand for meat and milk will double as the human population surpasses 9 billion by 2050. To meet this demand, livestock and poultry producers must continue to expand through sustainable intensification. Although the 'economies of scale' of intensive production improve efficiency and profitability, it is not without challenges. High density housing increases the transmission of infectious diseases. Energy demands for growth can be so high that the immune system is weakened and the susceptibility of herds and flocks to infectious disease increased. Antimicrobials are often added to the diet to reduce infectious diseases or used to treat livestock and poultry with clinical disease. These practices are increasingly being scrutinized by consumers with a number of retail and fast food chains developing 'antibiotic-free' meat and milk. In reality, regulations that ensure the production of 'antibiotic-free' meat and milk have been enforced for decades. However, free of antibiotics does not necessarily mean free of antimicrobial resistance (AMR) bacteria, as they are part of the natural microbial world both in the presence and absence of antimicrobials. Using antimicrobials in livestock and poultry production undoubtedly increases AMR, but linkages to those AMR bacteria that have the greatest impact on humans is less clear. The microbial world still harbors countless antimicrobials with therapeutic potential, but the private sector lacks the drivers needed to commercialize them. Until viable alternatives to antimicrobials are identified, it is important that science plays a key role in policy development that may further curtail their use in livestock and poultry production.

Résumé

La demande pour le lait et la viande doublera d'ici 2050, quand la population de la planète aura dépassé les 9 milliards d'individus. Pour répondre à cette demande, les producteurs de bétail et de volaille doivent continuer à se développer par le biais d'une intensification durable. Bien que l'« économie d'échelle » de la production intensive améliore l'efficacité et la rentabilité, cela demeure un véritable défi. La production animale en densité élevée favorise la transmission des maladies infectieuses. La demande en énergie pour la croissance peut être si forte que le système

immunitaire s'affaiblit et que la susceptibilité des troupeaux aux maladies infectieuses augmente. Des antimicrobiens sont souvent ajoutés à la ration pour réduire les maladies infectieuses ou sont utilisés pour traiter le bétail et la volaille contre les maladies cliniques. Ces pratiques sont de plus en plus remises en question par les consommateurs, ce qui encourage un certain nombre de chaînes d'alimentation au détail et de restauration rapide à offrir de la viande et du lait « sans antibiotiques ». En réalité, la réglementation entourant la production de viande et de lait « sans antibiotiques » est en vigueur depuis des décennies. Cependant, l'absence d'antibiotiques ne garantit pas l'absence de bactéries résistantes aux antimicrobiens, puisque celles-ci font partie de l'univers microbien naturel, avec et sans antimicrobiens. Il ne fait aucun doute que l'utilisation d'antimicrobiens en production animale favorise la résistance, mais les liens avec les bactéries résistantes aux antimicrobiens les plus importantes chez l'humain sont moins clairs. L'univers des microbes recèle encore d'innombrables antimicrobiens au pouvoir thérapeutique, mais le secteur privé manque de motivation pour les commercialiser. Jusqu'à ce que des solutions de remplacement viables soient identifiées, il est important que la science joue un rôle significatif dans l'élaboration des politiques qui pourraient restreindre davantage leur utilisation dans les systèmes de production de bétail et de volaille.

Introduction

Shortly after the discovery of penicillin by Sir Alexander Fleming in 1928, it was recognized that bacteria could become resistant to antibiotics. The emergence of antimicrobial resistance in bacterial pathogens is a serious global issue. Antimicrobial use in livestock and humans selects for antimicrobial-resistant (AMR) bacteria that reside in agricultural and clinical biomes. Besides pathogens, AMR bacteria include many harmless and beneficial microbes that act as a genetic reservoir of AMR gene determinants the so-called resistome (Martinez et al. 2015). These AMR genes can be transferred via various mechanisms of horizontal gene transfer (HGT) throughout the microbial community. With alarming frequency, untreatable human and animal pathogens with multiple AMR determinants are arising. The emergence of AMR in pathogens is commonly accepted as a result of widespread use and abuse of antimicrobials in agriculture and medicine. The use of antimicrobials in agriculture has attracted particular attention, in part due to the immensity of global meat production where antimicrobials are routinely used to support animal health, and controversially, to promote growth and production efficiency.

Consumer pressure to eliminate antimicrobials in livestock production

Antimicrobials have become an important management tool in intensive livestock and poultry production systems. Global models of future meat and milk needs indicate that intensification of livestock and poultry production systems will be an integral part of ensuring future food security for humanity. As a result, the level of antimicrobial use is most prominent in those regions of the world that have intensive production systems and high animal populations (Figure 1).

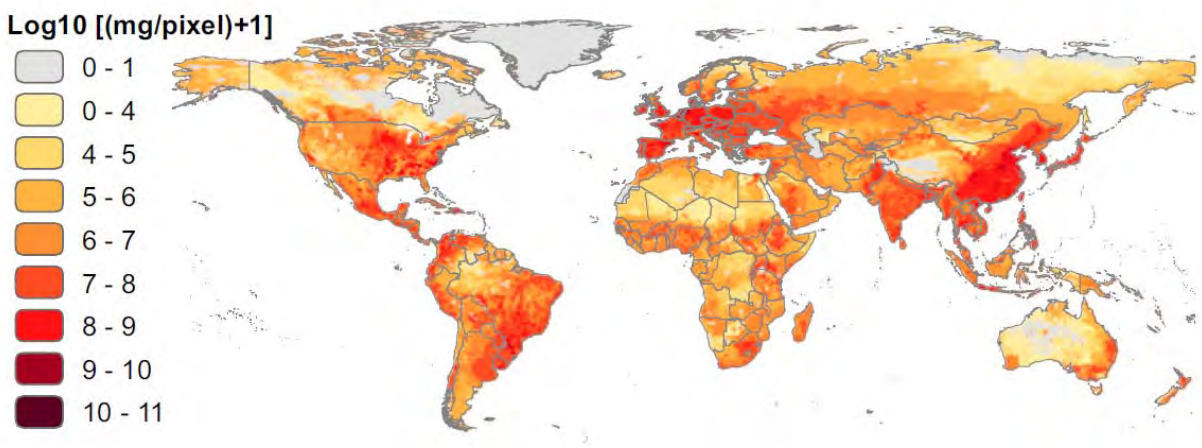

Figure 1. Global antimicrobial consumption in milligrams per 10 km^2. Note that regions of the world with the most intensive livestock production also have the highest antimicrobial use. (Van Boeckel et al. 2015).

Most of the pressure to eliminate antimicrobials has stemmed from the 'meat raised without the use of antibiotics movement.' Many fast food chains including Subway, Chipotle, A and W, Panera, Chick-fil-A and McDonalds have pledged to source meat from animals that are raised without antibiotics that are used in human medicine. In reality, meat itself seldom contains antibiotics, as in most countries producers must already adhere to strict dosing and withdrawal times before animals enter the food chain. With this in mind, the next possible benefit arising from 'antibiotic-free' production could be a reduction in the number of AMR bacteria that enter the food chain or broader environment.

The use of antibiotics inevitably results in an increase in AMR and although a reduction in antimicrobial use (AMU) in livestock and poultry production will in turn reduce AMR, it is unlikely to eliminate it. This is because genes coding for AMR are often linked to genes coding for other fitness traits such as tolerance to pH, temperature, metals or other environmental challenges. If these other selective pressures are present, a subgroup of the total bacterial population may still retain genes that code for AMR even in the absence of antimicrobials. Furthermore, some bacteria exhibit natural resistance to certain antibiotics as is the case for *Escherichia coli*, which are always resistant to the macrolides, tylosin, tilmicosin and tulathromycin. A recent study suggested that curtailing the use of antibiotics in food animals may have little impact on AMR in humans when transmission of AMR bacteria from humans to animals is high (Van Bunnik and Woolhouse 2017). In our work, we have observed that those enterococci species associated with infectious disease in humans are often absent or are only represent a minuscule fraction of the total enterococci population in the digestive tract of cattle. However, a lack of a reduction in the AMU in food animals is likely to make it equally challenging to achieve a significant reduction in AMR in humans. This is why any movement to limit AMU needs to be approached from a 'One Health' perspective where all aspects of AMU and the resulting consequences are considered. Simply lowering the AMU in food animals is unlikely to significantly lower AMR in humans unless transmission both from humans to animals and animals to humans is considered.

Antimicrobial resistance is a natural phenomenon

It is almost certain that antimicrobial resistance arose as a result of dynamic and competitive interactions among bacteria that pre-date the use of antibiotics by humans. Indeed, genes coding for antibiotic resistance have been identified in ancient DNA dating from the Pleistocene (D'Costa et al. 2011). Studies of members of a culturable microbiome collected from a cave isolated for 4 million years in New Mexico exhibited resistance to many of the same antimicrobials presently used in human and veterinary medicine (Figure 2)

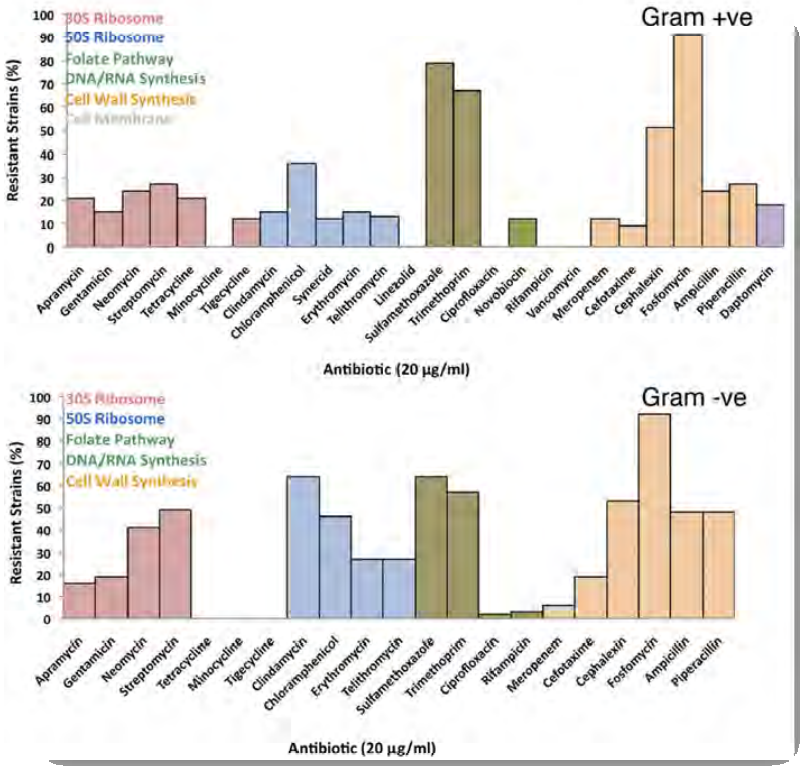

Figure 2. Resistance levels of cave bacteria against various antibiotics with the top figure showing Gram + and the bottom figure representing gram – bacteria. (Bhullar et al. 2012).

Antibiotics almost certainly play an important role in establishing the structure of microbial communities and influence their interactions with the environment. At high concentration this may be through overt interactions that inhibit the growth or kill bacteria. At low concentrations, antibiotics may simply be a form of cell to cell communication that ensures that microbial communities remain in sync with their environment through subtle changes in gene transcription (Figure 3).

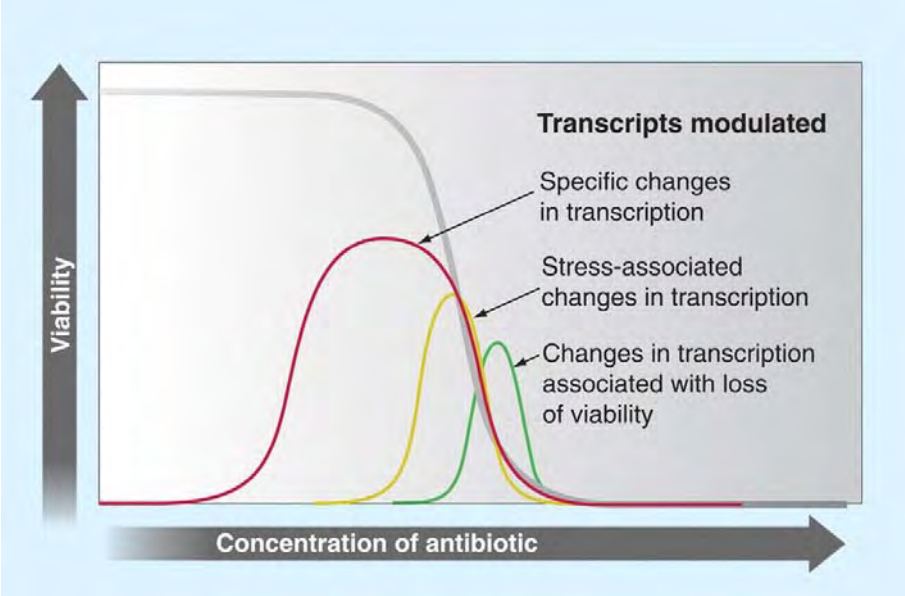

Figure 3. Effects of antibiotics on bacterial gene expression. Low concentrations of antibiotics may play a role in cell-to-cell signaling whereas high concentrations may cause substantial changes in transcription that lead to death of the bacterial cell. (Martinez et al. 2008).

Are there really no new antibiotics to be developed?

Most antibiotics are natural products derived from microbes that reside in soil and at this point only a minuscule fraction of these have been developed into commercial antibiotics. In the past, antibiotic discovery was limited to those microorganisms that could be grown in the laboratory under controlled conditions. This automatically eliminated the 99% of microbes in soil environments that could not be grown in the laboratory as potential sources of new and novel antibiotics. New approaches to culture bacteria within their natural soil environments could open the door to studying antibiotics produced by this previously untouchable population. This is how a new antibiotic known as 'teixobactin' was recently discovered by a group of collaborating scientists in the UK, Germany and the USA (Figure 4).

However, despite showing promise in mice, teixobactin is still likely to be at least 5 years away from clinical trials with humans assuming that no severe toxicological or adverse side effects are identified during pretesting.

Other scientists have discovered novel antibiotics, literally right under their nose. Recently a stain of *Staphylococcus lugdunensis* isolated from the nasal cavity of humans was found to produce a cyclic peptide antibiotic known as lugdunin, which prohibited colonization of the nasal cavity by Staphylococcus aureus (Zipperer et al. 2016) . These as well as other studies demonstrate that there are likely countless antimicrobials produced in natural microbial communities that could be developed into novel drugs if there is the impetus to do so.

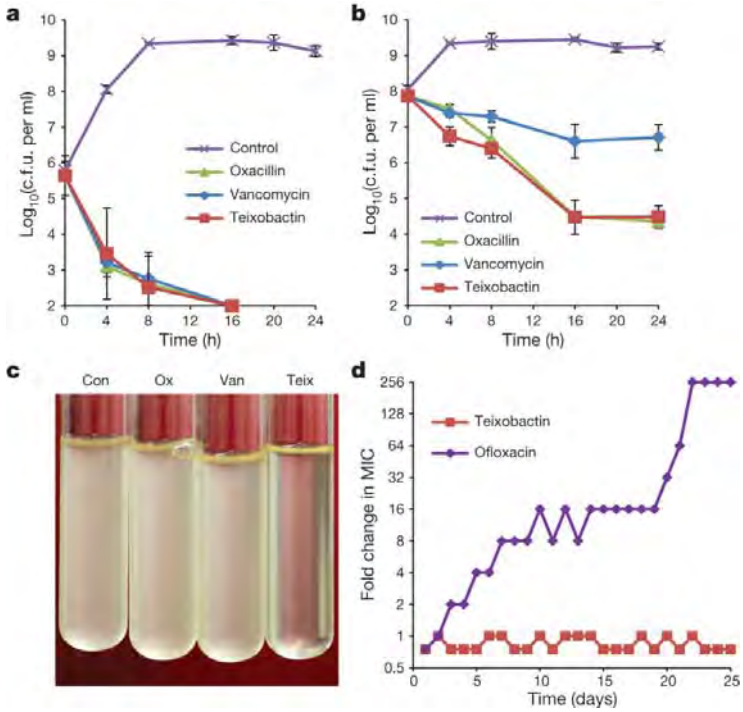

Figure 4. Time dependent killing of *Staphylococcus aureus* by teixobactin as compared to other antimicrobials (a,b,c) and a lack of resistance development after repetitive culture (d). (Ling et al. 2015).

Why are no new antibiotics being developed?

If potential sources of antibiotics have not been exhausted, why are new antibiotics not being developed? The number of new antibiotics being developed has declined precipitously since the early 1980's (Figure 5). Furthermore, those antibiotics that have been developed often inhibit or kill bacteria using the same mechanisms as their predecessors; reducing the time it takes for bacteria to develop resistance. On average, pharmaceutical companies spend $5 billion on the research and testing required to bring a new antibiotic to market. As more than 80% of drugs fail to meet efficacy or safety standards, pharmaceutical companies need to generate billions in sales to offset the cost of drug development. As pharmaceutical companies are ultimately answerable to shareholders, investments are often directed towards drugs that are more frequently used and do not lose their effectiveness to resistance. These include antidepressant, anti-inflammatory, anti-cancer and anti-cardiovascular disease drugs. Today, only six of the top 50 pharmaceutical companies are still pursuing research into the development of new antibiotics. Some governments are offering incentives to develop antibiotics and some small to medium biotech companies are taking up this pursuit. These small companies often attempt to target the most critical antibiotics with the hope that they can generate intellectual property that will be of interest to major pharmaceutical companies. Although this approach may have potential in human medicine, it is unlikely to gain traction in veterinary medicine where the use of antibiotics is already being curtailed by the 'antibiotic free' movement. Development of a business model to develop a drug that becomes increasingly less effective the more you use it presents a challenge for both human and veterinary medicine.

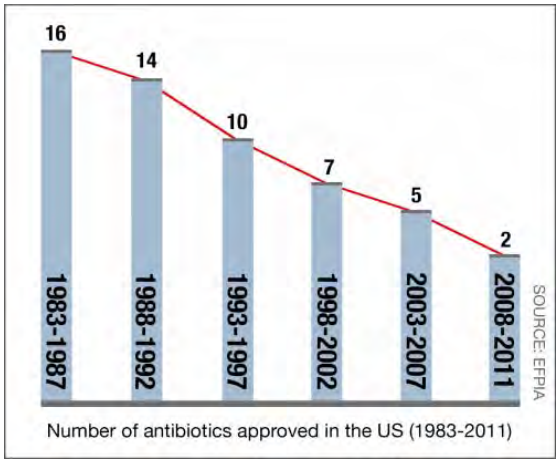

Figure 5. Decline in the number of new antibiotics approved in the US from 1983 to 2011. In recent years, development of new antibiotics has increased appreciably, despite the fact that some governments are offering incentives for development.

Conclusions

As in most environments, AMR determinants exist ubiquitously in livestock and poultry production biomes, regardless of antimicrobial exposure. Nevertheless, the use of antimicrobials to maintain the health and promote growth in food animals applies selective pressure that increases the abundance of AMR genes and antibiotic resistant bacteria. The connections between AMR in livestock and poultry microbial populations to human health are likely to become more apparent as antimicrobial use in food animal production continues to increase. Future investigations may validate mitigation strategies, such as the separation of the types of antimicrobials used in livestock and poultry from those used in humans. Proper and judicious use of antimicrobials will help prolong the usefulness of both clinical and veterinary antimicrobials. The pressure on reducing or even eliminating the use of antimicrobials in food animal production is likely to grow and if new antimicrobials are developed they will be more likely used exclusively in humans as opposed to veterinary medicine. As the number of effective antibiotics dwindles, antibiotics previously deemed unsuitable for use in humans (e.g., collstin), maybe resurrected further reducing the number of antibiotics available for use in veterinary medicine. Efforts to maintain those antimicrobials for use in veterinary medicine that do not pose a threat to human health or to develop alternatives, is essential to assuring that intensive food animal production can continue in a manner that fully meets animal welfare standards.

References

Bhullar, K., Waglechner, N., Pawlowski, A., Koteva, K., Banks, E. D., Johnston, M. D., Barton, H. A. and G. D. Wright. Antibiotic resistance is prevalent in an isolated cave microbiome. PLoS ONE 7(4):
e34953. doi:10.1371/journal.pone.0034953.

D'Costa, V. M., King, C. E., Kalan, L., Morar, M., Sung. W. W. L., Schwarz, C., Froese, D., Zazula, G., Calmels, F., Debruyne, R., Golding, G. B., Poinar, H. N. and G, D. Wright. 2011. Antibiotic resistance is ancient. 477: 457-461.

Ling, L. L., Schneider, T., Peoples, A. J., Spoering, A. L., Engels, I., Conlon, B. P., Mueller, A., Schäberle, T. F., Hughes, D. E., Epstein, S., Jones, M., Lazarides, L., Steadman, V. A., Cohen, D. R., Felix, C. R., Fetterman, K. A., Millett, W. P., Nitti, A. G., Zullo, A. M., Chen, C. and K. Lewis. 2015. A new antibiotic kills pathogens without detectable resistance. Nature 517: 455–459. doi:10.1038/nature14098

Martinez, J. L. 2008. Antibiotics and antibiotic resistant genes in natural environments. Science 321: 365-367.

Martínez, J. L., Coque, T. M. and F. Baquero 2015. What is a resistance gene? Ranking risk in resistomes. Nature Reviews Microbiology 13: 116-123.

Van Boeckel T.P., Brower, C., Gilbert, M., Grenfell B, T., Levin, S. A,, Robinson, T. P., Teillant, A. and R. Laxminarayan 2015 Global trends in antimicrobial use in food animals. Proc. Natl. Acad. Sci. USA 112: 5649-5654.

Van Bunnik, B. A. D. and M. E. J. Woolhouse. 2017. Modelling the impact of curtailing antibiotic usage in food animals on resistance in humans. R. Soc. Open Sci. 4: 161067. http://dx.doi.org/10.1098/rsos.161067.

Zipperer, A., Konnerth. M. C., Laux, C., Berscheid, A., Janek, D., Weidenmaier, C., Burian, M., Schilling, N. A., Slavetinsky, C., Marschal, M., Willmann, M., Kalbacher, H., Schittek, B., Brötz-Oesterhelt, H., Stephanie Grond, S., Peschel, A. and B. Krismer 2016. Human commensals producing a novel antibiotic impair pathogen colonization. Nature 535: doi:10.1038/nature18634

The Three Levels of Immunity in the Animal: A Case of a Good Defense Preventing Too Much Offense (and Damage)

Les trois niveaux d'immunité dans l'animal : le cas d'une bonne défensive évitant trop d'offensive (et de dommages)

Christopher C.L. Chase[1,]
[1]Professor of Veterinary Immunology and Virology, Department of Veterinary and Biomedical Sciences, South Dakota State University, Brookings, SD, 57007, christopher.chase@sdstate.edu

Abstract

Mucosal immune responses involve the immune modulation of the mucosa epithelial cells. These cells respond to microbial produced ligands and metabolites and modulate the immune response. They regulate anti-inflammatory response and regulatory environment to prevent overreaction of the pro-inflammatory response. Macrophages in the lamina propria are particularly sensitive to signals from gut bacteria including endotoxin from gram-negative bacteria to induce pro-inflammatory responses. With changes diet that occur at weaning or at parturition, the microflora population changes are considerable. This is where immune homeostasis is important-maintaining a balance between anti-inflammatory response of the mucosa and the pro-inflammatory response needed for the innate immune system to respond. This combination of adipose remodeling, macrophage activation and microflora can result in a cytokine storm. A cytokine storm (hypercytokinemia) is the systemic expression of a healthy and vigorous immune system resulting in the release of more than 150 known inflammatory mediators (cytokines, oxygen free radicals, and coagulation factors). It is an overreaction of the immune system. Both pro-inflammatory cytokines [such as tumor necrosis factor-alpha (TNF-alpha), interleukin-1, and Interleukin-6] and anti-inflammatory cytokines (such as interleukin 10 and interleukin 1 receptor antagonist) are elevated in the serum of people or animals experiencing a cytokine storm. In this case, a healthy immune system may be a liability rather than an asset. An experimental approach in cattle to study these interactions will be discussed along with the proof of concept immunological measurements. Studying specific localized responses will require novel approaches.

Résumé

La réponse immunitaire muqueuse est associée à une immunomodulation par les cellules épithéliales de la muqueuse. Ces cellules réagissent aux ligands et métabolites produits par les microbes et modulent la réponse immunitaire. En particulier, ils régulent la réponse anti-inflammatoire et les mécanismes de régulation afin d'éviter une réponse pro-inflammatoire exagérée. Les macrophages de la lamina propria sont particulièrement prompts à déclencher les

réponses pro-inflammatoires en réaction aux signaux émis par les bactéries intestinales, notamment l'endotoxine des bactéries à Gram négatif. À chaque changement de ration au sevrage ou à la mise bas, la microflore est considérablement perturbée. Dans ces moments, l'homéostasie est importante pour maintenir un équilibre entre la réponse anti-inflammatoire de la muqueuse et la réponse pro-inflammatoire dont le système immunitaire inné a besoin pour réagir. Ces situations entraînant en même temps un remodelage de tissus adipeux, l'activation des macrophages et le bouleversement de la microflore peuvent être à l'origine d'un choc cytokinique. Un choc cytokinique (hypercytokinémie) est l'expression systémique d'un système immunitaire sain et vigoureux provoquant la libération de plus de 150 médiateurs inflammatoires connus (cytokines, radicaux libres d'oxygène et facteurs de coagulation). Il s'agit d'une réaction exagérée du système immunitaire. La concentration de cytokines pro-inflammatoires (telles que le facteur de nécrose tumorale alpha [TNF-alpha], l'interleukine-1 et l'interleukine-6) et de cytokines anti-inflammatoires (telles que l'interleukine 10 et l'antagoniste des récepteurs de l'interleukine 1) est élevée dans le sérum des gens et des animaux souffrant d'un choc cytokinique. Dans un tel cas, un système immunitaire en santé peut être un handicap plutôt qu'un atout. Une approche expérimentale pour étudier ces interactions chez les bovins sera présentée ainsi que les valeurs immunologiques de preuve de concept. L'étude des réponses localisées spécifiques exigera des approches novatrices.

Introduction

The immune system consists of three lines of defense systems: barriers, innate immunity and adaptive or acquired immunity that work together to give cattle protection from disease (Figure 1). The barrier system is probably the most overlooked but it eliminates 99.9% of all infections. This system is very susceptible to dehydration and changes in microbial populations. The innate system is the first to be activated and responds almost immediately (Figure 2). The innate system has the job of sensing the host's environment – looking for infections and tissue damage. It then does its second job, which is to recruit in the "right" cells to handle the problem. The adaptive response follows up 10-14 days later in naïve animals but requires the innate system to kick into action. The immune system is regulated by anti-inflammatory response to prevent over response. The cumulative effect of these anti-inflammatory response is to suppress the immune system and to direct the immune response away from the memory response to the short-term antibody immune response. At the same time, over expression of pro-inflammatory cytokines from infectious agents, feed intake issues (acidosis, ketosis) and stress can result in immune dysfunction and an over reactive immune system that can result in immunopathology and disease (Sordillo 2016).

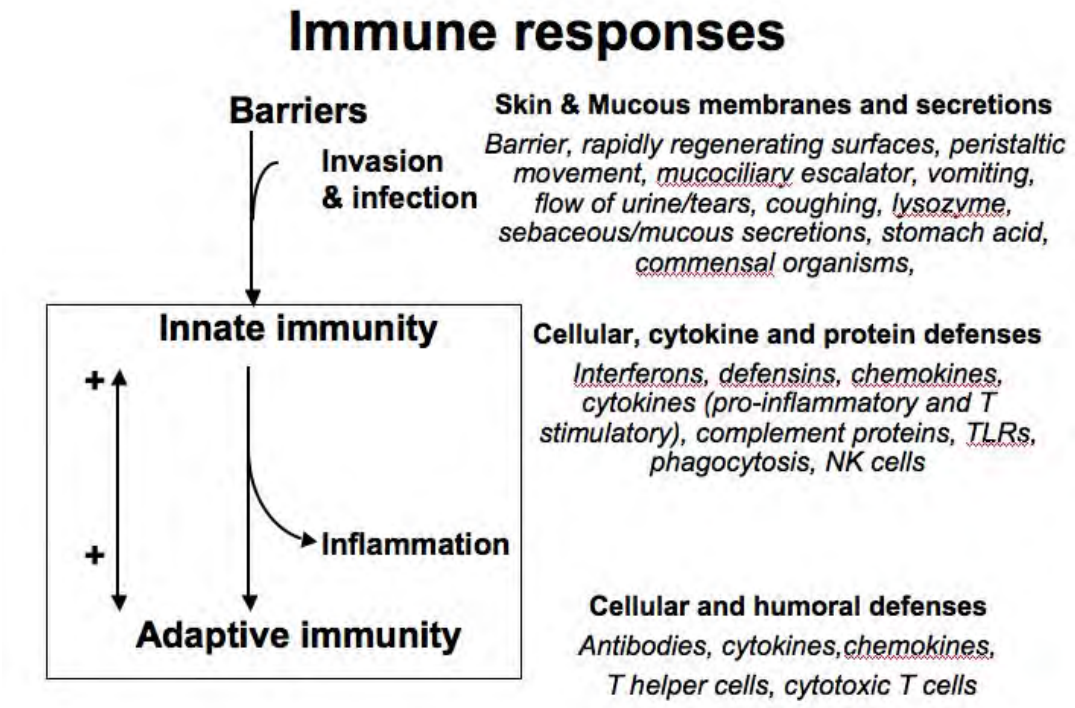

Figure 1. Immune responses: the barrier, innate, and adaptive immune components (courtesy of D. Topham, PhD, Rochester, NY, from Introduction to Viral Immunology: Part I).

Stress, Immunity, and Central Nervous System

There is ample evidence that both physical and psychological distress (**STRESS**) can cause immune dysfunction in animals, leading to an increased incidence of infectious disease (Salak-Johnson & McGlone, 2007). In cattle, there are several factors that will compromise immune function. There is the stress of transportation, dehydration, feed change (with the resulting negative energy balance), excess heat or cold, crowding, mixing, weaning, limit-feeding, parturition noise, and restraint are stressors that are often associated with intensive animal production and have been shown to influence immune function. Also, social status, genetics, age, and the duration of stress (chronic vs. acute) have been shown to be important in the animal's response to stress (Hulburt et al. 2016; Salak-Johnson & McGlone, 2007). The immune system and the central nervous system (CNS) are a bidirectionally linked "two-way street," each influencing the other (Borghetti et al. 2009). In particular, there is a critical balance that exists between hormones [growth hormone (GH), GCs, prolactin (PRL), catecholamines, and insulin], and the proinflammatory mediators (IL-1, IL-6, and TNF-α) of the immune system (Figure 3).

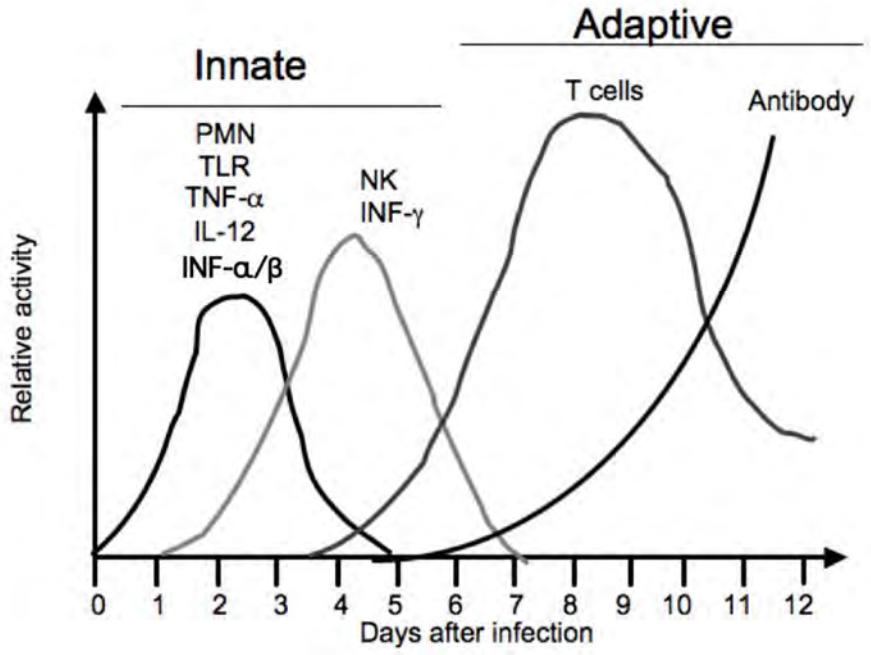

Figure 2. The timing of the host response to infection: mobilization of the innate and adaptive response. (courtesy of D. Topham, PhD, Rochester, NY, from Introduction to Viral Immunology: Part I). PMN-polymorphonuclear cell, neutrophil; TLR- toll-like receptors (pathogen recognition receptors); TNF-α- tumor necrosis factor alpha; -12-interleukin 12; IFN-α/β- interferon alpha or beta; IFN-γ- interferon gamma;); NK- Natural Killer

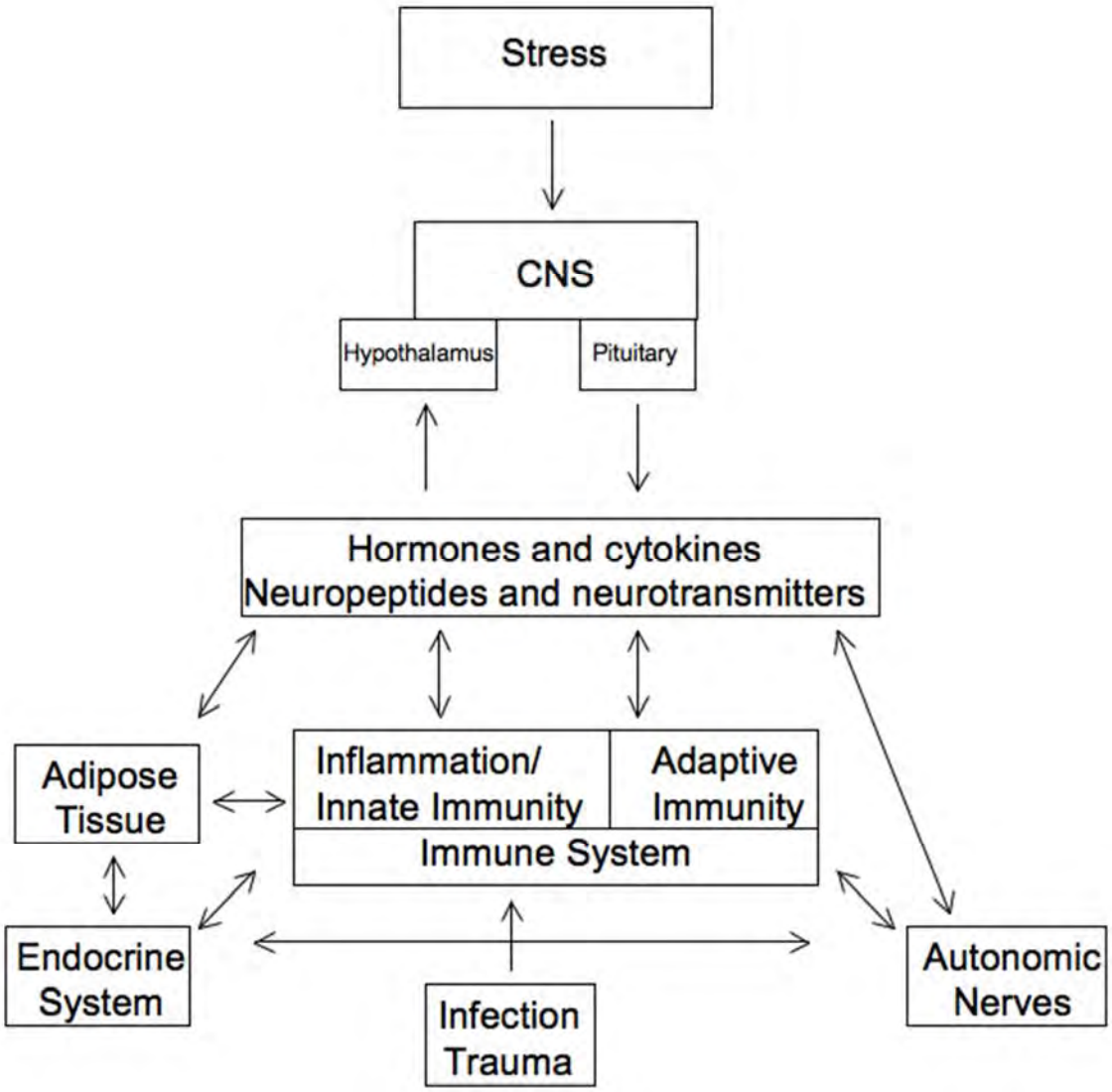

Figure 3. Stress effects on immunity. Network of bidirectional communication among central (CNS) and peripheral nervous systems, endocrine system, adipose tissue, and immune systems. Adapted from The Lancet Oncology, Reiche et al. (2004).

Immunity, Negative Energy Balance, Microflora and Cytokine Storm

The immune system is a major consumer of energy and in times of negative energy like seen in the newly weaned calf and the fresh dairy cow can be difficult times for the immune system to respond (Sordillo 2016). In addition, the mobilization of energy from adipose tissue (fat) results in infiltration of macrophages as activity of adipocytes (fat cells) results in inflammation. These macrophages are particularly sensitive to signals from gut bacteria including endotoxin from gram-negative bacteria (Winer & Winer, 2012). With changes in diet that occur at weaning or at parturition for the dairy cow the microflora changes are considerable changing populations. This combination of adipose remodeling, macrophage activation and microflora can result in a

cytokine storm (Figure 4) (Cluny et al 2012; Tisoncik et al. 2012). A cytokine storm (hypercytokinemia) is the systemic expression of a healthy and vigorous immune system resulting in the release of more than 150 known inflammatory mediators (cytokines, oxygen free radicals, and coagulation factors) (Tisoncik et al. 2012). It is an overreaction of the immune system. Both pro-inflammatory cytokines [such as tumor necrosis factor-alpha (TNF-alpha), interleukin-1, and Interleukin-6] and anti-inflammatory cytokines (such as interleukin 10 and interleukin 1 receptor antagonist) are elevated in the serum of people or animals experiencing a cytokine storm. It is believed that cytokine storms were responsible for many of the human deaths during the 1918 influenza pandemic, which killed a disproportionate number of young adults. In this case, a healthy immune system may have been a liability rather than an asset. Preliminary research results also indicated this as the probable reason for many deaths during the SARS epidemic in 2003 (Tisoncik et al. 2012). Human deaths from the bird flu H5N1 usually involve cytokine storms as well. Recent reports of high mortality among healthy young adults in the 2009 swine flu outbreak has led to speculation that cytokine storms could be responsible for these deaths, since the Swine Flu results from the same influenza strain as the Spanish Flu of 1918

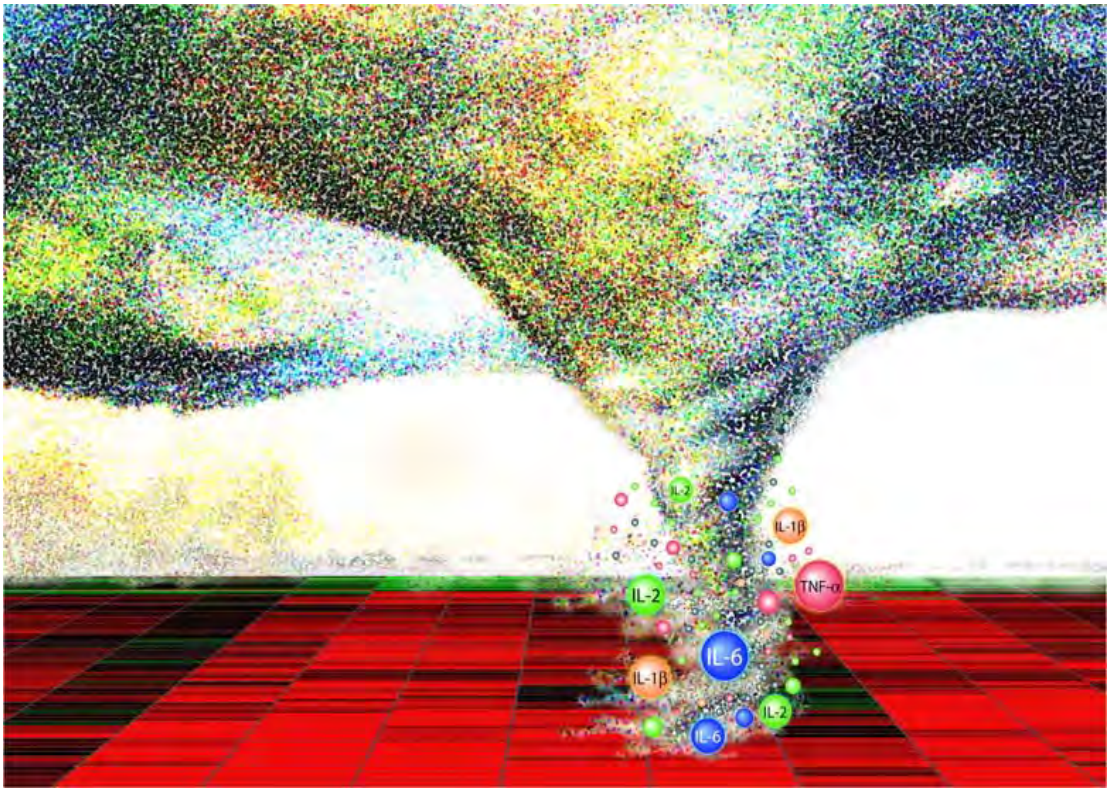

Figure 4. Imagery of a Cytokine Storm. Tisoncik JR et al. 2012.

Immunity in the Stressed Calf

In the stressed calf, there are several factors that will compromise immune function. There is the stress of transportation, dehydration, feed change (with the resulting negative energy balance), acidosis, and associated microbial changes in the gut (Hulburt et al. 2016). There is clear evidence that waiting at least 2 days and preferable as long as 2 weeks before vaccination

will result in better immunity and less sickness in that adjustment period after the stress (Richeson et al 2015). The issues that were described above for the activation of a cytokine storm are present in the stressed calf. The good news is that the macrophage activation in the stressed calf occurs over a much shorter period, so once the calves are back on feed and the microbiota is stable, the opportunity for the cytokine storm lessens dramatically.

Immunity in the Postpartum Period

The common practice of vaccinating during the fresh period (15-45 days in milk) is an immunological challenge for dairy cows due to the negative energy balance associated with the high-energy demands and the low dry matter intakes typically observed postpartum. The requirement of the immune system for energy becomes a secondary requirement compared to lactation

Depression in postpartum leukocyte function has been correlated to shifts in leukocyte trafficking patterns (Kehrli et al. 1989a,b; Nonnecke et al. 2003). The alteration in the proportions of the peripheral blood lymphocyte subset has been monitored in dairy cows during the pre-partum and post-partum periods. The variation of T cells was significant during the peripartum period, particularly around parturition. B cell and MHC II + populations remained constant until after calving and then decreased, returning to the initial subset proportion by Week 16**.** A decrease in the total number of T lymphocytes and changes in the T subpopulation have been reported in peripheral blood. In our research, we found that production, mastitis and reproductive health were improved in cows vaccinated in the prepartum period as compared to cows vaccinated in the postpartum period.

Approximately 30% of dairy cows suffer sub-clinical ketosis during the fresh period because of the negative energy balance (Suriyasathaporn et al. 1999). The pathogenesis of this phenomenon is explained by the metabolic changes that occur when nutrient intake, particularly energy, does not meet production demands. In high-producing cows this metabolic disorder usually occurs from a few days up to six weeks post-calving, with the highest incidence occurring at about three weeks post-partum. Most high-producing cows undergo sub-clinical ketosis in early lactation when they are unable to consume enough energy to meet demands. Cows in negative energy balance are utilizing body fat and protein stores because of a drop-in blood glucose concentration (glycemia). When fat molecules reach the liver, they are converted to ketones, and elevate ketone levels in the blood. High levels of blood ketone bodies in blood interfere with the production of T cells and impair the chemotactic response of leukocytes. There is a link between elevated ketone levels and the risk of mastitis. In addition, subclinical ketosis results in increased pro-inflammatory cytokine production enhancing the cytokine storm (Figure 4). Since sub-clinical ketosis is present in nearly 30% of fresh dairy cows suggests vaccination during this period is probably not the best approach and that vaccinating during the dry period might be a better alternative.

Evidence also exists that cows selected for high milk production traits, have an unfavorable correlated response in the functional capacity of immune functions traits. There is sufficient genetic variation in these immunological traits among sires of high genetic merit for milk production (Detilleux et al. 1999).

Another consequence of peripartum cows feed disorders is hepatic lipidosis, a consequence of the fat cow syndrome. Upon vaccination, over-conditioned cows have lower humoral and cellular response when compared with cows with a low liver triacylglycerol (TAG) at Day 14 after vaccination (Wentink et al. 1997). Cows in the transition period often face a challenge associated with low trace mineral levels. This is due to low dry matter intakes and stress, which causes excretion of trace minerals**.** Of concern are deficiencies in zinc, copper, chromium, manganese, cobalt, and selenium.

Summary

Management of the dairy cow and calves' immune system is not a simple process. Stressors and nutrition often compromise immunity including an overstimulation of the immune response. It is important that vaccinations be given at optimal times and that vaccination is not overused. Vaccination can never overcome poor management.

References

Borghetti, P., Saleri, R., Mocchegiani, E., Corradi, A., Martelli, P., 2009. Infection, immunity and the neuroendocrine response. Vet. Immunol. Immunopath. 130, 141–162. doi:10.1016/j.vetimm.2009.01.013

Cluny NL, Reimer RA, Sharkey KA. 2012. Cannabinoid signaling regulates inflammation and energy balance: The importance of the brain-gut axis. Brain, Behavior and Immunity. 26, 691-698.

Detilleux JC, Kehrli ME, Stabel JR, Freeman AE, Kelley DH. 1995. Study of immunological dysfunction in periparturient Holstein cattle selected for high and average milk production. Vet. Immunol. Immunopath. 44, 251–267. http://doi.org/10.1016/0165-2427(94)05302-9

Hulbert LE, **Moisá SJ**. 2016. Stress, immunity, and the management of calves. J. Dairy Sci. 99, 3199–3216. http://doi.org/10.3168/jds.2015-10198

Kehrli ME Jr, Nonnecke BJ, Roth JA. 1989a. Alterations in bovine neutrophil function during the periparturient period. Am J Vet Res. 50, 207-14.

Kehrli ME Jr, Nonnecke BJ, Roth JA. 1989b. Alterations in bovine lymphocyte function during the periparturient period. Am J Vet Res. 50, 215-20.

Nonnecke BJ, Kimura K, Goff JP, Kehrli ME. 2003. Effects of the mammary gland on functional capacities of blood mononuclear leukocyte populations from periparturient cows. J. Dairy Sci, 86, 2359–2368. http://doi.org/10.3168/jds.S0022-0302(03)73829-6

Reiche, E.M.V., Nunes, S.O.V., Morimoto, H.K., 2004. Stress, depression, the immune system, and cancer. Lancet Oncol. 5, 617–625. doi:10.1016/S1470-2045(04)01597-9

Richeson, J.T., Beck, P.A., Poe, K.D., Gadberry, M.S., Hess, T.W., Hubbell, D.S., 2015. Effects of administration of a modified-live virus respiratory vaccine and timing of vaccination on health and performance of high-risk beef stocker calves. The Bovine Practitioner 49, 37–42.

Salak-Johnson JL, McGlone JJ. 2007. Making sense of apparently conflicting data: Stress and immunity in swine and cattle. J. Ani. Sci. 85), E81–E88. http://doi.org/10.2527/jas.2006-538.

Suriyasathaporn W, Daemen AJ, Noordhuizen-Stassen EN, Dieleman SJ, Nielen M, Schukken YH. 1999. Beta-hydroxybutyrate levels in peripheral blood and ketone bodies supplemented in culture media affect the in vitro chemotaxis of bovine leukocytes. Vet. Immunol. Immunopath. 68, 177–186.

Sordillo LM. 2016. Nutritional strategies to optimize dairy cattle immunity. J. Dairy Sci. 99, 4967–4982. http://doi.org/10.3168/jds.2015-10354

Tisoncik JR, Korth MJ, Simmons CP, Farrar J, Martin TR, Katze MG. 2012. Into the eye of the cytokine storm. Micro. Mol. Biol. Reviews 76, 16–32. http://doi.org/10.1128/MMBR.05015-11

Wentink GH, Rutten VP, van den Ingh TS, Hoek A, Müller, KE, Wensing T. 1997. Impaired specific immunoreactivity in cows with hepatic lipidosis. Vet. Immunol. Immunopath. 56, 77–83.

Winer S, Winer DA. 2012. The adaptive immune system as a fundamental regulator of adipose tissue inflammation and insulin resistance. Immunol. Cell Biol. 90, 755-762. http://doi.org/10.1038/icb.2011.110

Measuring the Effects of Early Life Perturbations on Immune Development in Pigs and Poultry

Mesure des effets des perturbations en bas âge du développement immunitaire des porcs et des volailles

Dirkjan Schokker[1]
[1] Researcher on Gut Health, Wageningen University and Research Animal Breeding and Genomics, Wageningen, dirkjan.schokker@wur.nl

Abstract

Immediately after birth/hatch development of the gut occurs, this development can be categorized into morphological, functional, and immunological. Morphological changes include the increase of the villus height and crypt depth, which will contribute to a larger surface area of the gut. Another aspect is the functional development, i.e. the pH in each gut segment. The immunological development coincides with the colonization of the microbiota, their cross-talk results in the behavior of the host. For example, certain species reside in the gut and are beneficial for the host, so called symbionts, and are tolerated by the host. Contrary, when pathogens are observed by the immune system an immune response will be evoked. Thus, during the immune development this intimate interplay between the microbiota and the host is important. We have investigated the effects of early life perturbations on the gut system in several pigs and chicken studies. For pigs, i) administration of a single antibiotic at day 4, ii) fructooligosaccharides in the neonatal period of piglets, and for chicken, iii) administration of therapeutic dose of an antibiotic at day 1. In all these studies the host genome-wide expression profiling was performed, locally in the gut, as well as community-scale typing of the resident microbiota. This led to a better understanding of the underlying biological processes at the systems level. Because in many of these studies we gathered information on several 'biological levels', we also performed integration analysis in order to associate certain microbiota to distinct host biological processes. In conclusion, by better understanding the behavior of the gut as system, it will be possible to modulate the gut in a beneficial way, regarding immune competence (tolerance), or resilience against pathogens.

Résumé

Le développement intestinal débute immédiatement après la naissance ou l'éclosion. Ce développement peut être divisé en trois types, soit morphologique, fonctionnel et immunologique. Les changements morphologiques incluent l'augmentation de la hauteur des villosités et de la profondeur des cryptes, phénomène qui contribuera à accroître la surface intestinale. Un autre élément est le développement fonctionnel, c'est-à-dire le pH dans chaque segment intestinal. Le développement immunologique coïncide avec la colonisation du microbiote, les interactions entre les microbes étant responsables du comportement de l'hôte. Par exemple, certaines espèces résidant dans l'intestin sont bénéfiques pour l'hôte (les symbiotes) et sont tolérées par celui-ci. Au contraire, quand des agents pathogènes sont détectés par le système

immunitaire, une réponse immunitaire sera déclenchée. C'est pourquoi, durant le développement du système immunitaire, ce jeu d'interactions entre le microbiote et l'hôte est important. Nous avons examiné les effets des perturbations en bas âge sur le système intestinal répertoriés dans plusieurs études sur les porcs et les poulets. Pour les porcs, i) administration d'un antibiotique au jour 4, ii) fructooligosaccharides chez les porcelets nouveau-nés, et pour les poulets, iii) administration d'une dose thérapeutique d'un antibiotique au jour 1. Dans toutes ces études, le profilage d'expression pangénomique de l'hôte a été réalisé, au niveau local dans l'intestin, ainsi que le génotypage, au niveau de la communauté, du microbiote présent. Ces démarches ont permis une meilleure compréhension des processus biologiques systémiques. Puisque beaucoup de ces études nous ont fourni de l'information à plusieurs « niveaux biologiques », nous avons également effectué une analyse d'intégration nous permettant d'associer certains microbiotes aux processus biologiques d'hôtes spécifiques. En conclusion, une meilleure compréhension du comportement de l'intestin en tant que système permettra d'intervenir positivement sur l'intestin pour favoriser l'immunocompétence (tolérance) ou la résilience face aux agents pathogènes.

Introduction

The gut is the gatekeeper of health and approximately 70% of the immune cells are located in mucosal tissue. Furthermore, the gut has an important role for animal performance, i.e. feed efficiency and growth. Gut development occurs immediately after birth/hatch, and is categorized into morphological, functional, and immunological development. Morphological development includes the increase in villi height and crypt depth. In broilers this morphological development occurs mainly the first two weeks [1]. In piglets, gut maturation also occurs immediately after birth, however in piglets weaning has a large effect on the morphology, this transition from liquid feed to solid feed profoundly affects the villi height [2, 3]. In broilers, functional development, like acquiring a certain pH in an intestinal segment [4] and enterocyte maturation occur also in the first two weeks of life [5]. In pigs, in the first 8 weeks small fluctuations occur in the pH [6], however the small intestine doubles in size in the first 2-4 days. It has been shown that after the first colostrum intake the volume of enterocytes increase markedly [7]. Another process that occurs during development is the remodeling of the intestine, fetal type enterocytes gradually disappear in time and will be replaced by adult type enterocytes [8]. Lastly, immune development occurs coinciding with gut microbiota colonization. The cross-talk between host and microbiota, dominantly bacteria, will result in the programming of the immune system. When this process is disturbed/perturbed, it could affect the host in long-term. Evidence is accumulating that the period after birth/hatch is key to immune system and metabolic programming [9-15]. Also studies have shown that a higher risk to allergies and asthma occurs when the microbiota colonization is perturbed in early life [16-18]. Antibiotics have shown to be involved in the onset of obesity [19], as well as early life infections [20], this suggests that perturbations in early life have profound effects on the long-term for an individual.

Here, the effects of such early life perturbations are discussed in light of gut development, and in particular immune system programming. Three different studies will be discussed, for pigs the perturbations are, administration of a single antibiotic at day 4, or fructooligosaccharides in the neonatal period of piglets (day 2-14 of age), and for chicken administration of therapeutic dose of an antibiotic at day 1 will be discussed.

Three case-studies

The studies discussed here, are published elsewhere, therefore briefly the main results and conclusions from these studies.

i) Administration of a single antibiotic at day 4
At day 4 a single shot of antibiotic (Tulathromycin) was administered to one group of piglets, whereas control piglets only received a mock. Thereafter piglets were sacrificed at days 8, 55, and 176, in order to obtain intestinal tissue and luminal content. Day 8 shows the short-term effects of the treatment [21], whereas day 55 and 176 show the long-term effects [22]. For all these time-points whole genome transcriptomics and community scale microbiota data were generated to investigate the gut systems behavior.

At day 8 (short-term), an increase in the diversity was observed in antibiotic treated piglets compared to control piglets, as well as a different microbiota composition (measured by redundancy analysis). The changes induces by the antibiotic treatment were mainly affecting the average relative contribution (ARC) of early life colonizers, i.e. the ARC of anaerobic bacteria increased (*Bifidobacterium*, *Eubacterium*, *F. prausnitzii*, and *S. moorei*), contrary the ARC of facultative bacteria decreased (*S. aureus*). Besides the differences in microbial parameters, also a difference was observed in intestinal gene expression (i.e. jejunum and ileum). First significant probes were annotated, with these annotated genes a functional annotation clustering was performed, resulting in a top 5 of affected biological processes for jejunum and ileum. All the biological processes were of communicative nature and mostly involved in immune signaling (like chemokine and cytokine signaling). Based on these results the gene expression data was superimposed on two important immune signaling pathways, namely Toll-like Receptor signaling and Chemokine signaling. These showed lower gene expression in antibiotic treated piglets compared to their respective controls.

At day 55 (long-term), differences were only observed in gene expression, whereas at day 176 differences were mainly observed in microbial parameters. The gene expression differences observed at day 55 were more dominant in jejunum vs. ileum, when comparing antibiotic treated piglets to their respective controls. Up-regulated genes in ileum showed an enrichment in the biological process 'TNF/cytokine activity'. This result suggests that maybe the immune system programming was different at young age, and the gut system reacts differently to the newly acquired bacteria during the weaning period. Moreover, the microbial parameters at day 55 were not significantly different. As a consequence, the gut system of the antibiotic treated piglets develop into different homeostasis. At day 176, a significant difference was observed in both microbiota composition and diversity between antibiotic treated piglets and their respective controls. Since both groups of piglets were exposed to similar environmental conditions, these differences are driven by specific intrinsic animal factors. It could be the earlier mentioned difference in programming that leads to different homeostasis.

After these analyses per specific time-point (8, 55, ad 176), we hypothesized that certain biological processes are involved in these long lasting effects and are regulated by (molecular) factors [23]. To this end, we analyzed the data in two dimensions, treatment and time, by performing (quadratic) regression analysis. Thereafter, we conducted network-based data

integration in order to investigate correlations between host transcriptomics and gut microbiota. With this approach hubs were identified, hubs are highly connected regulators, for the long-term effects. In addition, certain bacteria were also associated to the temporal gene expression patterns. These results are again in line with the hypothesis of early life perturbation affecting the gut system long-term.

ii) Fructooligosaccharides in the neonatal period of piglets

This study is described in more detail at http://edepot.wur.nl/363190. Briefly, four sows with parity number 3 or 4 were used, and from day 109 until weaning sows were individually housed in conventional farrowing rooms. All sows had at least 14 piglets born alive and thereafter litters were limited to 14 piglets. Piglets were not given access to creep feed or a milk replacer during the lactation period. Half of the piglets (n=7) received fructooligosaccharides (FOS) by oral gavage, the other half (n=7) received water. This intervention was given from day 2 till 14, and at day2, 14, and 25 piglets were sacrificed in order to investigate the effects on the intestinal microbiota composition as well as the intestinal mucosal tissue.

At day 14 the colon microbiota composition differed between the piglets supplemented with FOS compared to the respective controls, and a so-called bifidogenic effect was observed. However these changes in microbial parameters did not alter the colonic gene expression. Contrary, for jejunum no significant changes were observed for composition at day 14 and 25. However, the diversity at day 25 was significantly different between FOS supplemented piglets compared to the controls. When investigating the gene expression in jejunum mucosa, we did observe significant changes at both days 14 and 25. At day 14, FOS supplemented piglets had decreased activity in cell cycle related processes compared to control piglets, and increased activity in extracellular matrix processes. At day 25, FOS supplemented piglets had decreased activity in immune related processes compared to the control piglets. Another observation was that morphological parameters, i.e. villi height and crypt depth, were significantly different at day 25, where FOS supplemented piglets had higher villi and deeper crypts compared to the respective controls.

Taken together, we showed a bifidogenic effect of FOS in colon, in addition differences in jejunal mucosal tissue were also observed. We speculate that this bifidogenic effect also leads to differences in jejunum by thus far unknown mechanisms.

iii) Administration of therapeutic dose of an antibiotic at day 1

In this study, broilers received a therapeutic dose of antibiotic (amoxicillin) is administered at day 1 to the drinking water for 24 hours, where the control group did not receive the antibiotic [15]. Broiler chicks were sacrificed at day 1 (n=80 controls), 5 (n=80 controls and n=80 antibiotic), and 14 (n=80 controls and n=80 antibiotic) in order to obtain intestinal tissue and luminal content. From these intestinal samples both transcriptomic activity was determined, as well as the microbiota composition. Microbial parameters were hardly modulated, i.e. diversity and composition, whereas the transcriptomic activity was significantly different between the antibiotic and control group. At day 5, the significant down-regulated genes in the antibiotic treated birds were dominantly associated to immune related processes, whereas up-regulated genes in these birds were involved processes such as extracellular matrix and cell development. At day 14, the changes from day 5 are not observed anymore, and most of the up-regulated genes

are associated to generic biological processes. Nevertheless, the low expression of genes involved in immune processes at day 5, may impact the immune system development occurring at that time. Therefore, we performed immunohistochemistry, i.e. staining specific immunological cells of interest, on $CD4^+$, $CD8^+$, and KUL-01^+ (macrophage-like) cells, in order to validate our hypothesis of the perturbed immune system development. $CD4^+$ and $CD8^+$ cells showed an increase in time, which was expected due to the maturing immune system (contact with antigens), however no significant differences were observed between antibiotic treated and control birds. Contrarily, the KUL-01^+ cells did show a significant difference at day 14 between antibiotic treated and control birds, where antibiotic treated birds had lower amounts of KUL-01^+ cells, in addition a numerical decrease was observed at day 5 for antibiotic treated birds.

In conclusion, these results are again in line with the hypothesis that immune system programming can be modulated by early life events.

General Conclusion(s)

Early life perturbations modulate immune system development of the host. When these perturbations have an antibiotic nature, it seems to negatively affect the host, lower expression of immune related genes/pathways and decreasing numbers of macrophage-like cells. A beneficial perturbation, i.e. FOS, showed some promising results, however the underlying (molecular) mechanisms are not yet known. By measuring multiple biological levels and time-points in individual experiments it became possible to integrate these data in order to gain a better understanding of the gut systems behavior. The latter will help in identifying new ways of modulating the host in a beneficial way, for example by management, genetics, and/or nutrition.

References

1. **Uni, Z., S. Ganot, and D. Sklan**, *Posthatch development of mucosal function in the broiler small intestine.* Poult Sci, 1998. **77**(1): p. 75-82.
2. **Cera, K.R., et al.**, *Effect of age, weaning and postweaning diet on small intestinal growth and jejunal morphology in young swine.* J Anim Sci, 1988. **66**(2): p. 574-84.
3. **Lalles, J.P., et al.**, *Gut function and dysfunction in young pigs: physiology.* Animal Research, 2004. **53**(4): p. 301-316.
4. **Nkukwana, T.T., et al.**, *Intestinal morphology, digestive organ size and digesta pH of broiler chickens fed diets supplemented with or without Moringa oleifera leaf meal.* South African Journal of Animal Science, 2015. **45**(4): p. 362-370.
5. **Uni, Z., et al.**, *Small intestinal development in the young chick: crypt formation and enterocyte proliferation and migration.* Br Poult Sci, 2000. **41**(5): p. 544-51.
6. **Hartman, P.A.**, et al., *Digestive Enzyme Development in Young Pig.* Journal of Animal Science, 1961. **20**(1): p. 114-&.
7. **Burrin, D.G., et al.**, *Porcine colostrum and milk stimulate visceral organ and skeletal muscle protein synthesis in neonatal piglets.* J Nutr, 1992. **122**(6): p. 1205-13.
8. **Skrzypek, T., et al.**, *Gradual disappearance of vacuolated enterocytes in the small intestine of neonatal piglets.* Journal of Physiology and Pharmacology, 2007. **58**: p. 87-95.
9. **Schokker, D., et al.**, *Early-life environmental variation affects intestinal microbiota and immune development in new-born piglets.* PLoS One, 2014. **9**(6): p. e100040.
10. **Schmidt, B., et al.**, *Establishment of normal gut microbiota is compromised under excessive hygiene conditions.* PLoS One, 2011. **6**(12): p. e28284.
11. **Wu, H.J. and E. Wu**, *The role of gut microbiota in immune homeostasis and autoimmunity.* Gut Microbes, 2012. **3**(1): p. 4-14.
12. **Cox, L.M., et al.**, *Altering the intestinal microbiota during a critical developmental window has lasting metabolic consequences.* Cell, 2014. **158**(4): p. 705-21.
13. **Round, J.L. and S.K. Mazmanian**, *The gut microbiota shapes intestinal immune responses during health and disease.* Nat Rev Immunol, 2009. **9**(5): p. 313-23.
14. **Mach, N., et al.**, *Early life establishment of the swine gut microbiome and impact on host phenotypes.* Environ Microbiol Rep, 2015.
15. **Schokker, D., et al.**, *Perturbation of microbiota in one-day old broiler chickens with antibiotic for 24 hours negatively affects intestinal immune development.* BMC Genomics, 2017. **18**(1): p. 241.
16. **Kozyrskyj, A.L., S. Bahreinian, and M.B. Azad**, *Early life exposures: impact on asthma and allergic disease.* Curr Opin Allergy Clin Immunol, 2011. **11**(5): p. 400-6.
17. **Russell, S.L., et al.**, *Early life antibiotic-driven changes in microbiota enhance susceptibility to allergic asthma.* EMBO Rep, 2012. **13**(5): p. 440-7.
18. **Russell, S.L., et al.**, *Perinatal antibiotic treatment affects murine microbiota, immune responses and allergic asthma.* Gut Microbes, 2013. **4**(2): p. 158-64.
19. **Cox, L.M. and M.J. Blaser**, *Antibiotics in early life and obesity.* Nat Rev Endocrinol, 2015. **11**(3): p. 182-90.
20. **Li, D.K., et al.**, *Infection and antibiotic use in infancy and risk of childhood obesity: a longitudinal birth cohort study.* Lancet Diabetes Endocrinol, 2017. **5**(1): p. 18-25.
21. **Schokker, D., et al.**, *Early-life environmental variation affects intestinal microbiota and immune development in new-born piglets.* PLoS ONE, 2014. **9**(6).
22. **Schokker, D., et al.**, *Long-lasting effects of early-life antibiotic treatment and routine animal handling on gut microbiota composition and immune system in pigs.* PLoS ONE, 2015. **10**(2).
23. **Benis, N., et al.**, *Network analysis of temporal functionalities of the gut induced by perturbations in new-born piglets.* BMC Genomics, 2015. **16**: p. 556.

Controlling Coccidiosis in Poultry Fed Diets Free of Anti-Cocci and Anti-Microbial Products

Contrôle de la coccidiose chez la volaille nourrie d'aliments sans produits anticoccidiose et antimicrobiens

Greg F. Mathis and Brett Lumpkins
[1] Southern Poultry Research, INC.,
ATHENS, GA 30607, southern_poultry_res@msn.com

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Abstract

Before the turn of the century, much of European society pushed livestock growers to discontinue the use of antibiotics in feed, which later resulted in a ban through the EU in 2006. Consequently, the pressure from US consumers has been mounting over the past several years to rear livestock animals without antibiotics in the feed. Through much of the life span of the integrated poultry industry, companies have turned to and relied on antibiotics (virginiamycin and bacitracins) and anti-coccidial drugs to maintain proper health and well being of the animals. In times past the US poultry industry had focused mainly on formulating diets at least cost possible, which lead to the production and use of exogenous feed enzymes in poultry diets. However, a change in the perception of US consumers immerged and the US poultry industry started to realize the possibility of a world without antibiotics in the feed or the use of ionophores for coccidial control. A presence of natural alternatives started to arise that was aimed at improving animal health from the gastrointestinal tract (GIT) of the animal. The GIT of the bird has been observed to be a very involved micro-ecosystem for a number of bacteria, many which are still unidentified. This focus on the intestinal community has lead to the development and use of a number of products such as probiotics, prebiotics, essential oils, organic acids, saponins and tannins to name a few, and will need to work with a coccidiosis vaccine program.

Résumé

Avant le début des années 2000, une bonne partie de la société européenne poussait déjà les éleveurs à cesser d'utiliser des antibiotiques dans les aliments du bétail, un mouvement qui a finit par conduire à une interdiction totale de cette pratique sur tout le territoire de l'UE en 2006. Cédant à cette impulsion, les consommateurs américains ont progressivement accentué la

pression depuis quelques années en faveur de l'élevage du bétail sans antibiotiques dans les aliments. Depuis l'intégration de la production, les entreprises avicoles ont pratiquement toujours compté sur les médicaments antibiotiques (virginiamycine et bacitracines) et anticoccidiens pour garder les animaux en santé et préserver leur bien-être. Par le passé, l'industrie avicole américaine a surtout été guidée par la formulation de rations au plus bas coût possible, ce qui a entraîné la production d'enzymes alimentaires exogènes et leur utilisation dans les rations pour volailles. Cependant, la perception des consommateurs américains a changé et l'industrie avicole du pays a commencé à entrevoir la possibilité d'un monde sans antibiotiques dans les aliments ou sans ionophores anticoccidiens. Des solutions de remplacement naturelles conçues pour améliorer la santé des animaux à partir de leur tube digestif ont commencé à apparaître. Le tube digestif s'est avéré un micro-écosystème très productif pour de nombreuses bactéries, dont beaucoup sont toujours non identifiées. L'importance accordée à cette communauté des microorganismes intestinaux a favorisé l'élaboration et l'utilisation de bon nombre de produits, tels que probiotiques, prébiotiques, huiles essentielles, acides organiques, saponines et tannins, entre autres, qui devront être utilisés en combinaison avec un programme de vaccination anticoccidienne.

Introduction

Coccidiosis continues to be the most frequently diagnosed disease in poultry. In fact coccidia are present in every poultry house in the world. All commercial poultry, at some time in their life, will become infected with coccidia. That's some ~9 billon coccidia infected chickens each year in the USA alone not counting layers, breeders, and turkeys. Coccidia are protozoan (*Eimeria spp*) parasites that infect the intestine or ceca of poultry. The level and species of coccidia will vary with anticoccidial programs, management, and bird age.

Several factors enable coccidia to spread rapidly and infect large numbers of confinement reared birds. All of the *Eimeria spp* infecting poultry have a rapid, approximately 7 day, life cycle and a high reproductive capacity. One *Eimeria* oocyst (external infective stage) can produce 10's of thousands of coccidial oocysts. In a poultry house where millions of oocysts are present it is easy to understand how quickly a clinical parasitic problem can occur. An oocyst is highly resilient to environmental conditions, including normal disinfectants that have no deleterious effect. Removing used litter, litter amendments, and fire flame treatment of floors can reduce oocyst level. However, due to a high reproductive capacity, the house will become contaminated again before the end of the bird's production period. Strict cleaning can actually change the timing and severity of coccidiosis compared to built-up litter facilities.

Every country or region of the world has the same *Eimeria* species with very little strain variation. *Eimeria maxima* reportedly has the most strain variation however the basic physiology, immunogenicity, and fecundancy are basically the same for all species. S. H. Fitz-Coy, (1992) reported that birds immunized with the coccidia vaccine, Coccivac-B, and independently challenged with 60 field coccidial isolates demonstrated substantial *E. acervulina*, *E. tenella* and *E. maxima* immunity, with 86% fully protected against the E. maxima isolates.

Polymerase Chain Reaction (PCR) technology uses the amplification of species-specific DNA sequences to determine the species of *Eimeria* present in poultry litter. Many researchers including M. Jenkins USDA, ARS, using PCR technology (Jenkins, M. et al., 2010) have shown that most poultry facilities (barns/ houses) generally have the major three species, *E. acervulina, E. maxima*, and *E. tenella* as well as a high prevalence of the minor species, such as *E. mitis* and *E. praecox*. Is this high prevalence due to just naturally occurring populations, increased incidence and level of anticoccidial drug resistance leading to reduced control for all species, or to increased use of vaccines that do not contain these species, thus no immunological protection to these species? What the consequences will be for increased incidence and higher numbers of these less pathogenic species is yet to be determined.

Impact of Coccidiosis in Poultry

Coccidiosis coupled with associated enteric bacterial issues makes it the most costly disease facing the poultry industry. A recent survey of southeastern USA poultry veterinarians ranked disease issues with Coccidiosis being number 1 and Necrotic Enteritis as number 2. Intestinal damage by Coccidiosis or other stressors (nutritional, environmental, etc.) enables *Clostridium prefringens* to proliferate and potentially cause Necrotic Enteritis. The most direct Necrotic Enteritis link is between *Clostridium prefringens* and coccidiosis with E. maxima being the leading cause. Mathis (Mathis and Hofacre, 2005) reproduced Necrotic Enteritis in chickens dosed with *Clostridium prefringens* and infected with *E. acervulina, E. maxima, E. necatrix*, or *E. brunetti*. Thus controlling or managing Coccidiosis and enteric bacterial infections are interrelated.

The goal of any anticoccidial program is to control severity of coccidiosis and also attempt to regulate when the primary damage will occur. The amount of damage is related to the species, amount, frequency and timing of exposure. Coccidiosis, even mild cases, have a negative impact on production with losses in feed conversion, weight gain, uniformity, pigmentation, and increased mortality. Teeter (2008) examined the effects of coccidiosis along several points in the growth of broilers using a calorimetric chamber. He found the detrimental effects of coccidiosis to be more pronounced as birds mature, especially during the major growth period, which occurs in the later phase of the grow-out. Birds with the same level of coccidiosis potentially could lose 9 g / day at 3 weeks of age vs 43 g/ day at 6-7 weeks of age. A major advantage with coccidia vaccines is that coccidiosis cannot be totally avoided, thus it is advantageous to have a controlled level of coccidiosis early (such as with a vaccine) so the birds can develop an adequate immunity and to allow compensatory performance gain to reach its fullest potential.

Eimeria are very immunogenic. With each cycle of coccidia in the host, immunological protection increases. The development of self –limiting immunity, which eventually protects a flock, is a very critical objective for a coccidiosis control program, whether vaccination or an anticoccidial drug program. Today poultry coccidiosis is controlled by the use of prophylactic feeding of anticoccidial drugs or vaccinating with live coccidial vaccines. Both types of programs rely on immunity development. In order to predict coccidiosis control and immunity development, performance and related oocysts litter/fecal numbers are valuable tools.

Anticoccidials are broadly divided into synthetic (or chemical) and ionophores. Fully sensitive chemical drugs limit oocysts shedding and related immunity development. The lack of full immunity once the chemical is removed influences subsequent degree and timing of coccidia development. Ionophores and partially resistant chemicals work similarly with partial direct control and regulated immunity development. Both of these traits allow some oocysts to be shed over the course of the growout with accompanying immunity development. Generally, oocysts shedding with this type program increases with a peak approximately day 28-35. However Chapman (1999) demonstrated that full immunity to most anticoccidial drugs take at least 6-7 weeks.

Coccidiosis Control in a Drug Free Rearing

Due to the increasing demand for drug-free birds and concerns of resistance issues with anticoccidials, the use of coccidiosis vaccination has grown tremendously in the last few years. The only method to produce a truly drug-free bird is through the use of coccidia vaccination. Vaccination programs use live oocysts, which are administered using a hatchery spray or gel, a gel puck placed into hatchery box, or in-ovo dosing. These methods provide a prescribed amount of oocysts at an early age enabling immunity development to progress rapidly but still at a desired rate. A significant amount of immunological protection develops by 14 days of age, allowing birds to withstand a substantial challenge by 21 to 28 days of age. Coccidial vaccines are of two types; non-attenuated (not altered) and attenuated. All vaccines contain at least *E. acervulina*, *E. maxima*, and *E. tenella*. Some contain *E. mivati, E. necatrix, E. brunetti*, and or *E. mitis*, and possibly more than one strain of *E. maxima*.

Non-attenuated vaccinated broilers' oocysts shedding starts with an early day 7 peak, a major peak days 18-28, and then a decline. Attenuation of the coccidia causes the attenuated vaccinated broilers to generally start oocysts shedding approximately a day earlier, with a lower oocysts shedding peak, and extends longer than non-attenuated vaccinated broilers. Many other factors influence oocysts shedding including management, duration of drug program, breed, vaccine condition and application. To sustain good coccidiosis management all programs and influences need to be considered.

The major concern with anticoccidial drugs is development of resistance. Resistance to some degree has developed to all drugs (Mathis and McDougald, 1982). Rotation and resting (not using for extended periods of time) slows resistance development. However with many of these drugs once resistance has developed it is very persistent / stable and years of non-use are needed to see a significant change. Anticoccidial sensitivity of coccidia isolated from poultry houses can be determined by Anticoccidial Sensitivity Tests. ASTs are very useful in attempting to predict the control program that will have the most useful/ sensitive drugs.

Some coccidial vaccines are comprised of drug sensitive strains. Vaccinating with vaccines that contain sensitive strains can potentially shift the coccidia population from a resistant to a more sensitive population (Mathis and Broussard, 2006). Coccidia isolated from 3 poultry complexes (8 farms each) were highly resistant to the drug Clinacox. After vaccinating with the coccidia vaccine, Coccivac-B, the coccidia were re-isolated. A major percentage of

coccidia were now sensitive, with one complex's coccidia isolates (all 8 farms) were 100 % sensitive to Clinacox. This is not a permanent change. However often performance and profits in production are improved with drugs after using a vaccine composed of drug sensitive coccidia strains.

Concentrating only on antibiotic free products, other than FDA approved anticoccidial drugs, there are very few products that meet the needed requirements for strong coccidiosis control. Several have demonstrated ability to reduce oocysts production; generally this has not translated into reduction in coccidial lesions or improved performance

One of the issues with coccidia vaccination is that it is live product that relies on immunity development thus birds are infected. Non-antibiotic products are often added to vaccination programs. Product, purity, dose level, and timing of the addition are critical. The two classes of products that have shown the most promise for helping control coccidia are the Saponin or Sapogenines (isolated from yucca or quillaja) and the Essential Oils (single EOS or blends). In a series of coccidia challenge studies, a yucca saponin product reduced *E. acervulina, E. maxima, E. tenella, E, brunetti*, and *E. mitis* compared to non-medicated infected controls (McDougald, L.R., 1982), and the strongest activity was seen against *E. tenella*.

In a coccidia challenge study with pure EO Oregano with a low coccidia mixed species challenge demonstrated reduction in lesion scores. An important criterion for a non-antibiotic product is not to interfere with coccidial immunity. Studies conducted with vaccinated birds given an oregano product displayed a significant reduction in oocysts cycling, which can be related to coccidia immunity development. This information suggested that it was not appropriate to use this product in the starter feeds with a vaccine. The oregano product has been shown to work in the grower feed with vaccination after immunity has had a chance to develop. A proprietary mixture of organic minerals, yeast cell wall oligosaccharides, and plant extracts has also been investigated (Duffy et al., 2005). This product showed significant anticoccidial activity however not at a level to disrupt coccidial immunity development. Thus this product demonstrated advantageous benefits when used continuously or only in the grower phase of a growout.

Even though coccidiosis always occurs, it can be managed. Using anticoccidial sensitivity tests to determine level of resistance will provide information on which drug has the best potential for usefulness. Key factors for drug usage are efficacy, sensitivity, timing of infection, and if producing antibiotic free birds. If managed properly vaccination programs can equal effectiveness and performance to a drug program. Key factors of vaccination are application, vaccine storage, and farm management. The use of non-antibiotic products in combination with vaccination has showed significant improvement in performance and a reduction in Necrotic Enteritis. Using the most effective drug program, including utilizing immunity and vaccination programs along with non-antibiotic products will provide successful coccidiosis management.

References

1. **Chapman, H. D.**, 1999. Anticoccidial drugs and their effects upon the development of immunity to Eimeria infections in poultry. Avian Pathol. 28:521-535.
2. **Fitz-Coy, S.H.**, 1992. Efficacy of Coccivac against field isolates of chicken *Eimeria,* VIIIth International Coccidiosis Conference, Palm Cove, Australia
3. **Jenkins, M., Klopp, S., Ritter, D., Miska, K., and Fetterer, R.** 2010. Comparison of Eimeria Species Distribution and Salinomycin Resistance in Commercial Broiler Operations Utilizing Different Coccidiosis Control Strategies, Avian Diseases Digest: Vol. 5, Issue 3, pg(s) 27-28
4. **Mathis, G. and Hofacre, C.,** 2005. Necrotic Enteritis association with Eimeria acervulina and E. maxima. The IXth International Coccidiosis Conference, Brazil pg. 188.
5. **Mathis, G. F. and Broussard, C**., 2006. Increased Level of Eimeria Sensitivity to Diclazuril After Using a Live Coccidial Vaccine. Avian Diseases 50.
6. **Mathis, G. F. and McDougald, L. R.,** 1982. Drug responsiveness of field isolates of chicken coccidia. Poultry Science 61:38-45.
7. **Teeter, R. G**., 2008. Calorimetry Applications Quantify the Variable Cost of Subclinical Coccidiosis ("Coccidiasis") at Various Points in the Broiler Growth Curve. Western Poultry Disease Conference Proceeding 2008: 99-101.

Managing Gut Function and Health in Non-Ruminants Without Antimicrobials: The Role of Nutrition

Gestion de la fonction et de la santé intestinales chez les monogastriques élevés sans antimicrobiens : Le rôle de la nutrition

M. Neijat, E. Kim, H. Leung and E. Kiarie
Department of Animal Biosciences, University of Guelph, Guelph, ON, N1G 2W1
ekiarie@uoguelph.ca

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Abstract

Diet formulation is a precise science driven by the need to match nutrient supply with requirements. Central to this proposition is an assumption of a functional gastrointestinal tract to digest and absorb nutrients and to excrete waste products. Instructively, the gut harbors a highly complex microflora made up of > 400 different species that are critical for the host nutrition, health, performance and quality of the products. However, there are many specific pathogens that also inhabit the gut and they generally cause dysfunctional gut when the ecosystem is disturbed in some manner. For example, there is greater risk of an outbreak of necrotic enteritis in poultry or post-weaning scours in piglets with the use of certain feed ingredients and nutrients. In the context of antimicrobial (AGP) free feeding program, nutrition has multi-factorial role for maintaining functional and healthy gut. For example, approaches that enhances digestive and absorptive capacity such as feed processing, feed enzymes and functional ingredients (epidermal growth factors, yeast nucleotides). It is also prudent to expect that pigs and poultry raised in AGP programs will experience higher incidences of intestinal health problems and prolonged period of immunological challenges. Thus strategies for suppressing pathogens and maintaining intestinal integrity such as probiotics, prebiotics, immune-nutrients and organic acids will also be integral for successful AGP free feeding programs. These approaches in combination with improvements in management, vaccination programs and/or genetics will be effective in optimizing growth performance of pigs and poultry raised without or with reduced antibiotics for growth promotion.

Résumé

La formulation de rations est une science guidée par la nécessité de combler les besoins en nutriments. L'atteinte de l'objectif suppose la présence d'un tube digestif fonctionnel pour digérer et absorber les éléments nutritifs et excréter les déchets. Cependant, le tube digestif comporte une microflore très complexe constituée de plus de 400 espèces de microorganismes différents et qui joue un rôle déterminant dans l'alimentation, la santé et la performance de l'hôte ainsi que dans la qualité de sa production. Il s'y trouve aussi de nombreux agents pathogènes spécifiques qui provoquent généralement des problèmes intestinaux quand l'écosystème est plus ou moins perturbé. Par exemple, l'utilisation de certains ingrédients alimentaires et nutriments augmente les risques de flambée d'entérite nécrotique chez la volaille. Dans le contexte des programmes d'alimentation sans antibiotiques promoteurs de croissance, la nutrition joue un rôle multifactoriel pour le maintien de la fonction et de la santé intestinales. Mentionnons par exemple les approches qui favorisent la digestion et l'absorption, comme la transformation des aliments et l'utilisation des enzymes alimentaires et ingrédients fonctionnels (facteurs de croissance épidermique, nucléotides de levure). De plus, on peut raisonnablement s'attendre à une plus forte incidence de problèmes de santé intestinale et à de plus longues périodes d'exposition du système immunitaire chez les porcs et volailles élevés sans antimicrobiens. Par conséquent, les stratégies permettant de retarder le développement des agents pathogènes et de maintenir l'intégrité intestinale, comme l'utilisation de probiotiques, d'immuno-nutriments et d'acides organiques, feront aussi partie intégrante du succès d'un programme d'alimentation sans antimicrobiens. Ces approches, jumelées aux améliorations apportées à la régie, aux programmes de vaccination ou à la génétique, seront efficaces pour optimiser la performance de croissance des porcs et des volailles élevés dans un contexte de réduction ou d'abandon des antibiotiques promoteurs de croissance.

Introduction

The primary functions of the gastrointestinal tract (GIT) are to digest and absorb nutrients and to excrete waste products (Pluske et al., 1996). A functional gut is a result of the status of many interrelated elements such as villi architecture, gut-associated immune system and microbiome (Figure 1). However, microbial status is very critical to the overall health and functionality of the gut. The GIT microbiome is responsible for a plethora of functions including intestinal development and functionality (as evidenced by differences seen between gnotobiotic and conventional animals), nutrient digestion and absorption, mucus secretion, immune development and cytokine expression (Klasing, 2007). However, there are many specific bacterial pathogens that also inhabit the GIT, and they generally cause disease when the gut ecosystem is disturbed in some manner. One question that generally arises in relation to the GIT microbiome, and particularly the area of gut ‘health’, is: what is ‘normal’ when referring to the health of the chicken or pig gut? (Hillman, 2004) suggested that emphasis should be placed on an ‘optimal’ GIT microbiota rather than a ‘normal’ microbiota being present, because it is very difficult to define what is ‘normal’ given the wide array of conditions animals are grown under.

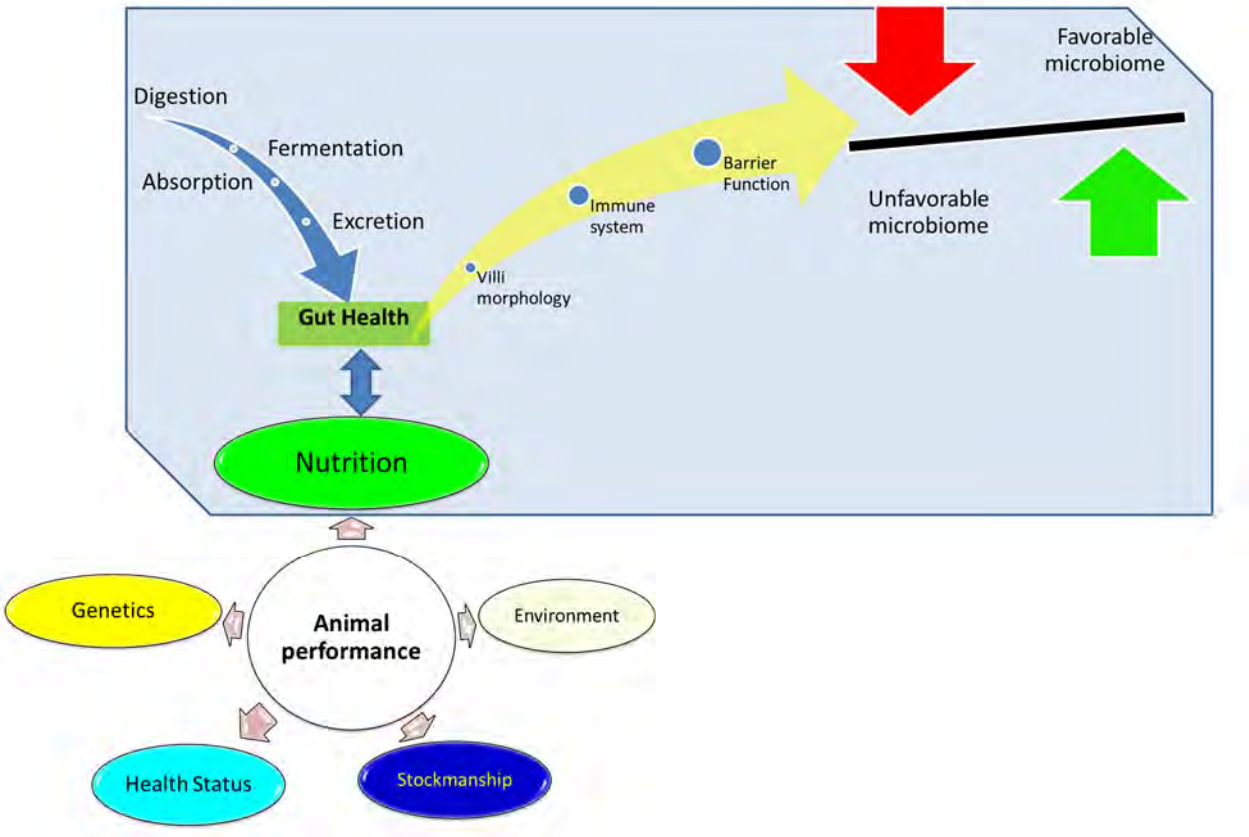


Figure 1. Several factors determine zootechnical performance (growth, reproduction) of an animal. Optimal nutrition is dependent on a functional and healthy gut and the intestinal microbiome is central to this network.

Producers strive to keep chickens and pigs free of infections (bacteria, viruses, parasites) to achieve the best utilization of feed for muscle gain and egg production as possible. However, with at least 400 species of bacteria, with numbers as high as 10^{14} colony forming units/g inhabiting the GIT (Savage, 1977), it is little wonder that perturbations sometimes occur to cause clinical disease and occasionally death (Kiarie et al., 2013). Specific enteric pathogens can cause enormous economic losses to poultry and swine enterprises, hence there is interest in being able to identify, quantify and track the different components of the microbiota (both pathogenic and non-pathogenic) to improve health and production. Many factors influence the diversity and activity of the GIT microbiota, including the age of the animal and the environment it inhabits, antimicrobial agents (antibiotics, anti-coccidials and minerals such as Zn and Cu), diet composition (e. g. type and content of carbohydrates and protein), feed additives (e.g. organic acids; feed enzymes), feed form, disease load, season, stress and genetics (Danzeisen et al., 2011; Kiarie et al., 2013).

Dietary strategies for managing metabolic realities of a living gut

The capacity for the intestine to absorb and assimilate nutrients may pose a proximal constraint upon the rate of growth of newly hatched chicks (Mitchell and Smith, 1991; Croom et al., 1999; Sklan et al., 2003; Gilbert et al., 2007). Studies have shown that at post-hatch, the small intestine of the hatchling accounts for a larger percentage of whole body weight (Wijtten et al., 2012). This developmental pattern is believed to reflect a survival strategy in which great importance is placed on the growth of nutrient supply functions early in life in order that post-absorptive growth functions can be maximized later in life cycle (Lilja, 1983; Ferket, 2012). In this context, nutritionists perceive functional and healthy gastrointestinal tract as a potential rate-limiting factor in the survival and productivity of poultry. This perception has been fostered by the emergence of ideas concerning the development and function of the digestive tract in the light of advances in genetic improvement and restriction on the use of antimicrobial growth promoters (**AGP**) and anticoccidial drugs. They are: 1) embryonic and immediate post-hatch periods represent a significant time-frame for attaining slaughter weight yet digestive and absorptive processes are not fully developed for metabolic programming of switching from yolk-based nutrition pre-hatch to carbohydrates and amino acids rich nutrition post-hatch (Nir et al., 1993; Sklan and Noy, 2003; Gilbert et al., 2007; Richards et al., 2010); 2) maternal antibodies are increasingly playing significant protective role since adaptive immune systems are not fully developed up two weeks post hatch (Bar-Shira and Friedman, 2005; Friedman et al., 2012); 3) restriction of antibiotics at hatchery and at placement is disruptive to microbial succession required for the establishment of positive microbiota balance and mucosal immune system development (Apajalahti et al., 2004; Kamada and Nunez, 2013; Stanley et al., 2013), and 4) GIT accounts for a large share of the animals total nutrients needs, and animals raised without AGP will experience prolonged inflammatory responses due to enteric pathogens exerting additional demands for nutrients usage for immune protection (Cant et al., 1996; Iseri and Klasing, 2014).

I. Stimulating functional gastrointestinal 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 for chicks and piglets to enhance digestive capacity and resilience to enteric pathogens (Kiarie, 2016). For example, epidermal growth factors, yeast nucleotides and diet structure. We recently completed a broiler trial evaluating the role epidermal growth factor (EGF) in stimulating growth performance and intestinal indices in broilers challenge with Eimeria (Kim et al, ANCC 2017). EGF is made up of 53 amino acids single chain polysaccharide and it is a critical component of mammalian colostrum and milk with a broad range of bioactivities on the intestinal epithelium, including stimulation of cellular proliferation, differentiation, and intestinal maturation in neonates (Jaeger et al., 1990; Playford and Wright, 1996). Previous work in our lab and others had shown gut health and function benefits of supplemental exogenous EGF in neonate or weaned mammals (James et al., 1987; Barnard et al., 1995; Kang et al., 2010). Based on this background, we fed broiler chicks EGF to assess its ability to stimulate gastrointestinal growth and function in broiler chicks post-hatch and how this relates to growth performance (Kim et al, ANCC, 2017. As shown in Figure 2, increasing dose of EGF improved day 5 body weight gain in broilers and was seen to improve expression of

genes for nutrient transporters and tight junction proteins in *Eimeria* challenged birds whilst no effect in non-challenged control (Kim et al., ANCC 2017.

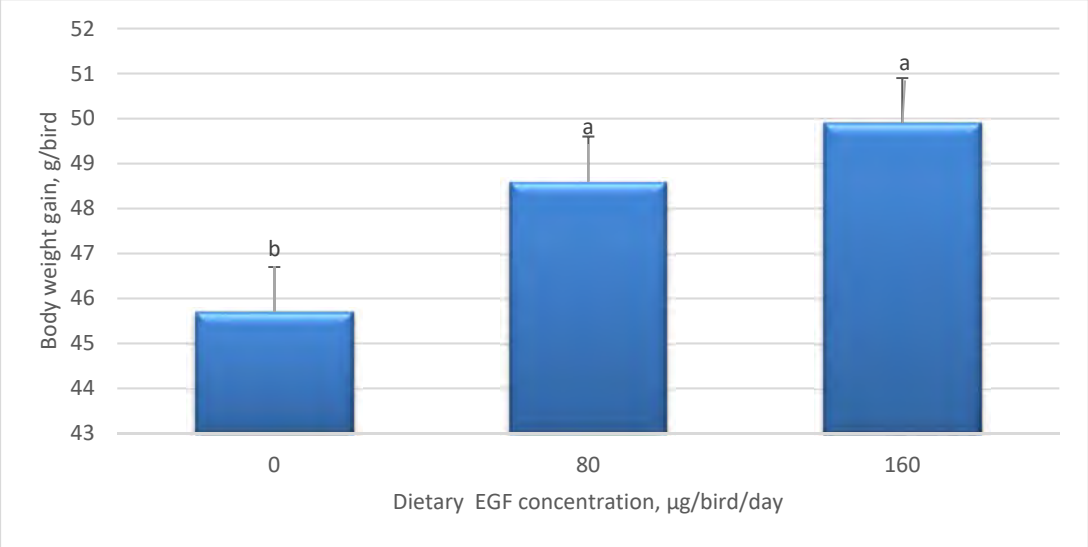


Figure 2: Effects of feeding EGF to broiler chicks on body weight gain from hatching to 5 days of life (Kim et al., ANCC 2017)

Nucleotides are the building blocks of the DNA and RNA molecules and are involved in structural, metabolic, energetic, and regulatory functions at the cellular level.(Uauy et al., 1990). We recently evaluated ability of yeast nucleotides (Max Gen Plus, CBS) to reduce the negative effects of *Eimeria* challenge on growth performance and indices of gut health and function (Leung et al., ANCC 2017). An interaction was observed between yeast nucleotides and *Eimeria* on jejunal villi height (VH) such that nucleotides fed birds had higher (533 vs. 447 µm) VH than birds not fed nucleotides upon challenge. Birds fed nucleotides had higher body weight gain (178 vs. 158 g) and a trend for lower FCR (1.09 vs. 1.29) compared to control birds. These data extended other studies (for example, (M'Sadeq et al., 2015), showing that Eimeria challenge resulted in growth depression and intestinal structural damage and supplemental yeast nucleotides improved intestinal histomorphology and growth post-challenge.

Unlike pigs, poultry requires a certain amount of diet structure for proper gut development and functionality (Hetland et al., 2005; 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). However, the diets of modern poultry have evolved such that the composition, ingredients choices and processing have been refined to improve intake and efficiency. The implications of these strategies are diets with low fiber and overall structure with negative consequences on the development and function of the gut (Mateos et al., 2012). For example, investigations on replacing (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 for broilers found that birds fed diets containing 25 and 50% course corn exhibited increased BW, improved FCR, and increased apparent ileal digestibility of energy and N, linked due to enhanced gizzard development (Xu et al., 2015) 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

It has been estimated that about 400 to 450 kcal/kg of energy is not digested in broilers fed a standard corn-soy diets corresponding to 15% undigested protein and fat and more than 50% undigested phosphorous (Cowieson, 2010; Kiarie et al., 2015). It is inevitable that the use of any additive that influences the digestibility of the diet will change the selection pressures on the resident microbiota which in turn will moderate the efficiency with which the host utilizes its feed. Beneficial effects of feed enzymes are inextricably linked to the amount of the undigested fat, protein and starch in the ileum. (Romero et al., 2013; Romero et al., 2014). Accelerated intestinal digestion and removal of what would otherwise be apparently undigested without feed enzyme must clearly limit the nutrients available for the microbes. Indeed, (Torok et al., 2008) used the terminal restriction fragment length polymorphism method to examine changes in gut microbial communities in response to the addition of a fiber degrading enzyme product containing β-glucanase, xylanase and protease activities in a barley-based diet. The enzyme product improved growth performance and energy utilization compared with the control. Further correlation analysis of apparent metabolizable energy and microbial community composition within the ileum and caeca revealed distinct clusters associated with control and enzyme-supplemented birds. Further research in this area have shown correlation between supplemental feed enzymes, nutrients utilization and gut microbiota (Munyaka et al., 2016). Studies linking differences in the composition of the gut microbial community with improved performance, imply that the presence of specific beneficial and/or absence of specific detrimental bacterial species may contribute to the improved performance in birds receiving feed enzymes. Furthermore, these studies are clear indication that molecular techniques coupled with statistical methods are capable of identifying desirable and undesirable clusters of organisms as far as good performance is concerned. Previous reports showed that supplemental carbohydrases reduced caecal counts of *Campylobacter jejuni* in broilers (Fernandez et al., 2000) and *Brachyspira intermedia* in the laying hen (Hampson et al., 2002). Clearly, supplemental feed enzymes might have dramatically altered the caecum ecology to the extent that for these specific bacteria growth was not favorable.

III. Low crude protein synthetic amino acids supplemented diets

In poultry and pig 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 as detrimental (Nyachoti et al., 2006; Heo et al., 2013). 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

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. (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 is the most critical period of a chick life. The newly hatched chick is susceptible to environmental and health challenges due to undeveloped digestive, thermoregulatory and immune system. For example, chicks are hatched in a clean environment, and unlike other farm animals, there is essentially no exposure to healthy microflora of adults. Colonization of mucosal surfaces in newly hatched chickens is therefore dependent on environmental exposure mainly through feeds, litter, water etc. Recent evidence suggest early intervention at this stage in life impacts who colonize the gut in terms of microbiota with implications to long term bird health, performance and safety of the products for human consumption (Pedroso et al., 2013). 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). The seed of neonatal gut microbiota originate from the parent and therefore steps to control gut health should start at the parent flock level. Vertical transmission of gut inhabitants (from parent to offspring) can be transovarial (inside the egg) or as a result of contamination during oviposition (Pedroso et al., 2013). In the artificially clean hatchery environment, even low doses of beneficial bacteria can significantly improve resistance to pathogen colonization, and artificial seeding of the gut at an early age has been shown to be beneficial (Owings et al., 1990; Edens et al., 1997). 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).

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, so as to avoid pathogen proliferation. There are many feed additives that could be used to promote proliferation of beneficial microbiome (Patterson and Burkholder, 2003). Some oligosaccharides, such as inulin and oligofructose, 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)). Manufactured sources of oligosaccharides may allow the dose to be more precisely defined and controlled, but only a very limited range of oligosaccharide structures is presently available. Most are linear, with short chain length and containing only one or two different sugar units, and are specially manufactured for the ever increasing human market. Plant and plant co-products used by the feed industry contain almost an unlimited range of polysaccharides (Table 1) (Kiarie et al., 2013).

Table 1. Monomeric sugar composition of enzyme hydrolysis products from common feed stuffs (Kiarie et al., 2013)

Sugar Type, mg/g	Feed ingredient			
	Soybean Meal	Canola Meal	Wheat Midds	Flaxseed Meal
Arabinose	17.6	81	108	46
Xylose	2.9	39.1	208	196
Mannose	8.4	nd	7.9	nd
Galactose	129	25	15.5	69.2
Glucose	32.8	42.7	293	113.5

Nd=not detected.

The use of specific fiber degrading enzyme, singly or in combination, against a range of polysaccharides can generate very large numbers of oligomer mixtures (Kiarie et al., 2013). In this context, short-chain xylo-oligosaccharides (AXOS) derived from the *in vitro* hydrolysis of wheat bran with an endoxylanase and fed to broiler chickens resulted in increased bifidobacteria populations in the caecum and improvements in feed conversion ratio (FCR) on both wheat- and wheat-based diets (Courtin et al., 2008). These data indicated that AXOS modulated gut health by increasing bifidobacteria counts in line with observations in other trials (Kiarie et al., 2009b; Damen et al., 2011; Onrust et al., 2015). 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).

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 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 commercial regulated products (Oakley et al., 2014). Alternative strategies have capitalized on increasing knowledge of the molecular basis that the pathogens use to attach to the mucosal to colonize. 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 of the gut (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 (Corrigan et al., 2015). We have shown that hydrolyzing feedstuffs with fiber degrading enzymes generate oligosaccharides capable of attenuating enterotoxigenic *E. coli* in pigs (Kiarie et al., 2013). Some studies reported that pathogen-specific hen egg antibodies can confer protection to pigs experimentally challenged with enterotoxigenic *E. coli* (Marquardt et al., 1999; Kiarie et al., 2009a), however success was not seen in broilers fed hen antibodies against *C. perfringens* or cholera toxin (Wilkie et al., 2006).

Take home message

Raising poultry and swine successfully without AGP will be challenging and will require several approaches and strategies "*no silver bullet*" for optimizing gut health and performance. Diet composition and formulation strategies will be pivotal for the development and maintenance of a functional and healthy gut and so are other elements such as management and genetics (Figure 3). Innovations of alternative dietary

strategies for maintaining bird health and productivity will be imperative for profitability and sustainability of the poultry and swine industries under restricted use of antimicrobials.

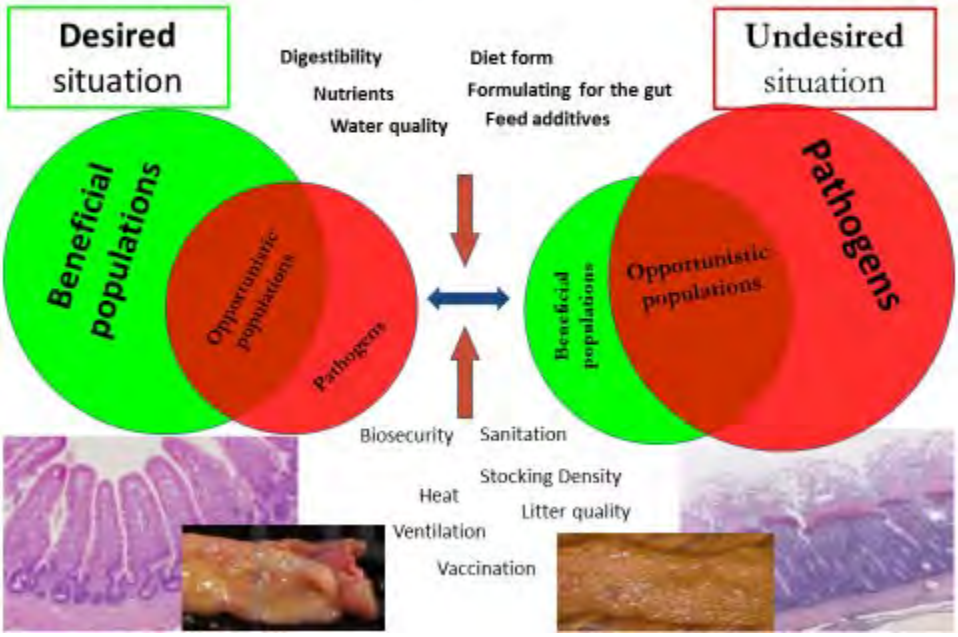


Figure 3. Strategies for maintaing a functional gut in the context of AGP feeding programs

References

Apajalahti, J., A. Kettunen, and H. Graham. 2004. Characteristics of the gastrointestinal microbial communities, with special reference to the chicken. World Poult. Sci J. 60: 223-232.

Bar-Shira, E., and A. Friedman. 2005. Expression of innate immune functions in developing broiler gut associated lymphoid tissue in the immediate pre and posthatch period. Poult. Sci. 84: 27-27.

Barnard, J. A., R. D. Beauchamp, W. E. Russell, R. N. Dubois, and R. J. Coffey. 1995. Epidermal growth factor-related peptides and their relevance to gastrointestinal pathophysiology. Gastroenterology 108: 564-580.

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: 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: 3460-3470.
Courtin, C. M. et al. 2008. Dietary inclusion of wheat bran arabinoxylooligosaccharides induces beneficial nutritional effects in chickens. Cereal Chem. 85: 607-613.
Cowieson, A. J. 2010. Strategic Selection of Exogenous Enzymes for Corn/soy-based Poultry Diets. J. Poult. Sci 47.: 1-7.
Croom, W. J. et al. 1999. Is intestinal absorption capacity rate-limiting for performance in poultry? J. Appl. Poultry Res. 8: 242-252.
Damen, B. et al. 2011. Prebiotic effects and intestinal fermentation of cereal arabinoxylans and arabinoxylan oligosaccharides in rats depend strongly on their structural properties and joint presence. Mol. Nutr. Food. Res. 55: 1862-1874.
Danzeisen, J. L., H. B. Kim, R. E. Isaacson, Z. J. Tu, and T. J. Johnson. 2011. Modulations of the chicken cecal microbiome and metagenome in response to anticoccidial and growth promoter treatment. PLoS One 6: e27949.
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: 414-420.
Edens, F. W., C. R. Parkhurst, I. A. Casas, and W. J. Dobrogosz. 1997. Principles of ex ovo competitive exclusion and in ovo administration of Lactobacillus reuteri. Poult. Sci 76: 179-196.
Ferket, P. R. 2012. Embryo epigenomic response to breeder management and nutrition XXIV World's Poultry Congress. p 1-11, Salvador, Bahia, Brazil.
Fernandez, F., R. Sharma, M. Hinton, and M. R. Bedford. 2000. Diet influences the colonisation of Campylobacter jejuni and distribution of mucin carbohydrates in the chick intestinal tract. Cell Mol. Life Sci. 57: 1793-1801.
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: 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: 1401-1412.
Gilbert, E. R., H. Li, D. A. Ernmersonj, K. E. Webb, and E. A. Wong. 2007. Developmental regulation of nutrient transporter and enzyme mRNA abundance in the small intestine of broilers. Poult. Sci 86: 1739-1753.
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: 361-379.
Hampson, D. J., N. D. Phillips, and J. R. Pluske. 2002. Dietary enzyme and zinc bacitracin reduce colonisation of layer hens by the intestinal spirochaete Brachyspira intermedia. Vet. Microbiol. 86: 351-360.
Heo, J. M. et al. 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: 207-237.
Hetland, H., B. Svihus, and M. Choct. 2005. Role of insoluble fiber on gizzard activity in layers. J. Appl. Poultry Res. 14: 38-46.
Hillman, K. 2004. An analysis of gut microbes Pig inter. No. 34. p 27-39.

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.
Jaeger, L. A., C. H. Lamar, T. R. Cline, and C. J. Cardona. 1990. Effect of Orally-Administered Epidermal Growth-Factor on the Jejunal Mucosa of Weaned Pigs. Am. J. Vet. Res. 51: 471-474.
James, P. S., M. W. Smith, D. R. Tivey, and T. J. G. Wilson. 1987. Epidermal Growth-Factor Selectively Increases Maltase and Sucrase Activities in Neonatal Piglet Intestine. J. Physiol-London 393: 583-594.
Kamada, N., and G. Nunez. 2013. Role of the gut microbiota in the development and function of lymphoid cells. J. Immunol. 190: 1389-1395.
Kang, P. et al. 2010. Epidermal Growth Factor-Expressing Lactococcus lactis Enhances Intestinal Development of Early-Weaned Pigs. J. Nutr. 140: 806-811.
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: 71-88.
Kiarie, E., B. A. Slominski, D. O. Krause, and C. M. Nyachoti. 2009a. Acute phase response of piglets fed diets containing non-starch polysaccharide hydrolysis products and egg yolk antibodies following an oral challenge with Escherichia coli (K88). Can. J. Anim. Sci .89: 353-360.
Kiarie, E., B. A. Slominski, D. O. Krause, and C. M. Nyachoti. 2009b. Gastrointestinal ecology response of piglets fed diets containing non-starch polysaccharide hydrolysis products and egg yolk antibodies following an oral challenge with Escherichia coli (K88). Can. J. Anim. Sci. 89: 341-352.
Kiarie, E., T. Woyengo, and C. M. Nyachoti. 2015. Efficacy of New 6-Phytase from Buttiauxella spp. on Growth Performance and Nutrient Retention in Broiler Chickens Fed Corn Soybean Meal-based Diets. Asian-Australas J. Anim. Sci. 28: 1479-1487.
Klasing, K. C. 2007. Nutrition and the immune system. Br Poult Sci 48: 525-537.
Lilja, C. 1983. A Comparative-Study of Postnatal-Growth and Organ Development in Some Species of Birds. Growth 47: 317-339.
M'Sadeq, S. A., S. B. Wu, M. Choct, R. Forder, and R. A. Swick. 2015. Use of yeast cell wall extract as a tool to reduce the impact of necrotic enteritis in broilers. Poult. Sci 94: 898-905.
Marquardt, R. R. et al. 1999. Passive protective effect of egg-yolk antibodies against enterotoxigenic Escherichia coli K88+ infection in neonatal and early-weaned piglets. FEMS Immunol. Med. Microbiol. 23: 283-288.
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: 156-174.
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: 328-331.
Mitchell, M. A., and M. W. Smith. 1991. The effects of genetic selection for increased growth rate on mucosal and muscle weights in the different regions of the small intestine of the domestic fowl (Gallus domesticus). Comp. Biochem. Physiol. A Comp. Physiol. 99: 251-258.

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: 528-540.
Nir, I., Z. Nitsan, and M. Mahagna. 1993. Comparative Growth and Development of the Digestive Organs and of Some Enzymes in Broiler and Egg Type Chicks after Hatching. Brit. Poult. Sci. 34: 523-532.
Nurmi, E., and M. Rantala. 1973. New aspects of Salmonella infection in broiler production. Nature 241: 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: 125-134.
Oakley, B. B. et al. 2014. The chicken gastrointestinal microbiome. FEMS Microbiol Lett 360: 100-112.
Onrust, L. et al. 2015. Steering Endogenous Butyrate Production in the Intestinal Tract of Broilers as a Tool to Improve Gut Health. Front Vet. Sci. 2: 75.
Owings, W. J., D. L. Reynolds, R. J. Hasiak, and P. R. Ferket. 1990. Influence of Dietary Supplementation with Streptococcus-Faecium M-74 on Broiler Body-Weight, Feed Conversion, Carcass Characteristics, and Intestinal Microbial Colonization. Poult. Sci. 69: 1257-1264.
Patterson, J. A., and K. M. Burkholder. 2003. Application of prebiotics and probiotics in poultry production. Poultr Sci 82: 627-631.
Pedroso, A. A. et al. 2013. Can Probiotics Improve the Environmental Microbiome and Resistome of Commercial Poultry Production? Int. J. Env. Res. Pub. He. 10: 4534-4559.
Playford, R. J., and N. A. Wright. 1996. Why is epidermal growth factor present in the gut lumen? Gut 38: 303-305.
Pluske, J. R. et al. 1996. Maintenance of villus height and crypt depth, and enhancement of disaccharide digestion and monosaccharide absorption, in piglets fed on cows' whole milk after weaning. Br. J. Nutr. 76: 409-422.
Richards, M. P., M. Proszkowiec-Weglarz, R. W. Rosebrough, J. P. McMurtry, and R. Angel. 2010. Effects of early neonatal development and delayed feeding immediately post-hatch on the hepatic lipogenic program in broiler chicks. Comp. Biochem. Phys. B 157: 374-388.
Romero, L. F., C. M. Parsons, P. L. Utterback, P. W. Plumstead, and V. Ravindran. 2013. Comparative effects of dietary carbohydrases without or with protease on the ileal digestibility of energy and amino acids and AME(n) in young broilers. Anim. Feed Sci. Technol. 181: 35-44.
Romero, L. F. et al. 2014. Contribution of protein, starch, and fat to the apparent ileal digestible energy of corn- and wheat-based broiler diets in response to exogenous xylanase and amylase without or with protease. Poult. Sci. 93: 2501-2513.
Savage, D. C. 1977. Microbial ecology of the gastrointestinal tract. Annu. Rev. Microbiol. 31: 107-133.
Sklan, D., S. Heifetz, and O. Halevy. 2003. Heavier chicks at hatch improves marketing body weight by enhancing skeletal muscle growth. Poult. Sci. 82: 1778-1786.
Sklan, D., and Y. Noy. 2003. Functional development and intestinal absorption in the young poult. Brit Poult. Sci. 44: 651-658.

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.
Sunkara, L. T. et al. 2011. Butyrate enhances disease resistance of chickens by inducing antimicrobial host defense peptide gene expression. PLoS One 6: e27225.
Torok, V. A., K. Ophel-Keller, M. Loo, and R. J. Hughes. 2008. Application of methods for identifying broiler chicken gut bacterial species linked with increased energy metabolism. Appl. Environ. Microbiol. 74: 783-791.
Uauy, R., G. Stringel, R. Thomas, and R. Quan. 1990. Effect of dietary nucleosides on growth and maturation of the developing gut in the rat. J. Pediatr. Gastroenterol. Nutr. 10: 497-503.
Waititu, S. M. et al. 2014. Effect of supplementing direct-fed microbials on broiler performance, nutrient digestibilities, and immune responses. Poult. Sci 93: 625-635.
Wijtten, P. J. A., D. J. Langhout, and M. W. A. Verstegen. 2012. Small intestine development in chicks after hatch and in pigs around the time of weaning and its relation with nutrition: A review. Acta Agriculturae Scandinavica, Section A - Anim. Sci. 62: 1-12.
Wilkie, D. C., A. G. Van Kessel, T. J. Dumonceaux, and M. D. Drew. 2006. The effect of hen-egg antibodies on Clostridium perfringens colonization in the gastrointestinal tract of broiler chickens. Prev. Vet. Med. 74: 279-292.
Xu, Y. et al. 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. Poult. Sci. 94: 53-60.

Growth Performance and Indices of Gut Function and Health in Broiler Chickens Fed Corn-Soybean Meal Diets with or without Exogenous Epidermal Growth Factor upon Challenge with *Eimeria*

Rendement de croissance et indices de fonction intestinale et de santé chez les poulets à griller nourris au maïs et au tourteau de soja, sans et avec facteur de croissance épidermique exogène en présence d'Eimeria

E. Kim, N. Akhtar*, J. Li*, J. Barta †, Y. Yang‡, C. Yang‡, and E. Kiarie**

**Dept. of Animal Biosciences, University of Guelph, Guelph, ON, N1G 2W1*
† Dept. of Pathobiology, University of Guelph, Guelph, ON, N1G 2W1
‡Dept. of Animal Science, University of Manitoba, Winnipeg, MB, R3T 2N2
ekiarie@uoguelph.ca

Abstract

Epidermal growth factor (EGF), a potent stimulant of gastrointestinal growth was assessed in broiler chickens challenged with *Eimeria*, a causative agent for coccidiosis. A total of 216 d old male broiler chicks were placed in cages (6 birds/cage) and allocated to three treatments. The treatments were: 1) control (fermentation media without EGF), 2) 80 µg of EGF/kg BW/d and 160 µg of EGF/kg BW/d. A basal antibiotic-free corn-soybean diet was offered with the respective treatments for 14 d. On d 5, birds (6 replicates per treatment) were challenged with 1 mL of *E. acervulina* and *E. maxima* via oral gavage and the other 6 replicates were given sham. Two birds per cage were sacrificed on d 10 for intestinal samples. Excreta samples were collected from d 10-13 and all birds were killed on d 14 for gastrointestinal weight. EGF linearly ($P < 0.05$) increased BWG in the pre-challenge period. There was no EGF and *Eimeria* interaction ($P > 0.05$) on growth, DM retention and intestinal histomorphology, however, *Eimeria* depressed ($P < 0.01$) performance, DM retention, and resulted in high intestinal lesion scores. An interaction between EGF and *Eimeria* ($P < 0.05$) on indices of gut function was such that EGF improved expression of digestive enzymes and nutrient transporters, toll-like receptor 4 and tight junction proteins in *Eimeria* challenged birds whilst no effect in non-challenged birds. *Eimeria* challenge reduced growth performance and impaired gut function while EGF showed beneficial effects on pre-challenge growth and improved indices of gut function upon *Eimeria* challenge.

Résumé

Le facteur de croissance épidermique (EGF), un puissant stimulant de la croissance gastro-intestinale, a été évalué chez les poulets à griller exposés à Eimeria, un agent responsable de la coccidiose. Au total, 216 poussins à griller mâles de un jour ont été placés dans des cages (6 oiseaux/cage) et répartis entre les trois traitements suivants : 1) témoin (milieux de fermentation sans EGF), 2) 80 µg d'EGF/kg de poids corporel/j et 160 µg d'EGF/kg de poids corporel/j. Une

ration maïs-soya de base sans antibiotiques a été servie avec les traitements respectifs pendant 14 jours. Au jour 5, les oiseaux (6 répétitions par traitement) ont été exposés avec 1 mL de E. acervulina et E. maxima administré oralement par gavage et un traitement fictif a été administré dans le cas des 6 autres répétitions. Deux oiseaux par cage ont été sacrifiés au jour 10 et des échantillons intestinaux ont été prélevés. Des échantillons d'excréments ont été recueillis des jours 10 à 13 et tous les oiseaux ont été abattus au jour 14 pour mesurer le poids gastro-intestinal. Une augmentation linéaire ($P < 0,05$) du gain de poids pour la période précédant l'exposition a été observée chez les oiseaux traités avec l'EGF. Aucune interaction entre l'EGF et Eimeria ($P > 0,05$) n'a eu d'effet sur la croissance, la rétention de MS et l'histomorphologie intestinale, bien que Eimeria ait été associée à une diminution ($P < 0,01$) de la performance et de la rétention de MS ainsi qu'à des cotes de lésions intestinales élevées. Une interaction entre l'EGF et Eimeria ($P < 0,05$) sur les indices de fonction intestinale a été observée et a permis d'établir que l'EGF a amélioré l'expression des transporteurs d'enzymes digestives et de nutriments, des récepteurs Toll 4 et les protéines associées à la jonction des tissus chez les oiseaux exposés à Eimeria, tandis qu'aucun effet n'a été observé chez les oiseaux non exposés. L'exposition à Eimeria a diminué la performance de croissance et a nuit à la fonction intestinale; l'EGF a eu des effets bénéfiques sur la croissance avant l'exposition et sur les indices de fonction intestinale après l'exposition à Eimeria.

Introduction

Coccidiosis is a significant disease in the global poultry industry, accruing more than $6 billion dollars in morbidity and mortality losses (Chapman, 2014; Chapman *et al.,* 2016). Characterized by the invasion of the host's intestinal cells by the Apicomplexian protozoa *Eimeria,* the resulting damage to the intestinal mucosa impairs nutrient digestion and absorption, gut barrier function, and ultimately leads to bacterial infections such as necrotic enteritis (Chapman, 2014). In light of the advancements in genetic improvement and the restrictive use of antibiotic growth promoters and anti-coccidial drugs, it is believed that a dysfunctional gastrointestinal tract is the potential determining factor in the survival and productivity of poultry (Roberts *et al.,* 2015). As feed costs constitutes a major percentage of overall production cost in broiler production, poor feed conversion as a result of an inefficient or impaired gastrointestinal tract, translates into extensive economic losses for the producer (Roberts *et al.,* 2015).

Epidermal growth factor (EGF) is a single chain polypeptide comprised of 53 amino acids, and a critical component of mammalian colostrum and milk with a multitude of bioactivities on the intestinal epithelium, including stimulation of cellular proliferation, differentiation, and intestinal maturation in neonates (Jaeger *et al.,* 1990; Playford and Wright, 1996). Interestingly, the EGF receptor is found along the length of the intestine on both luminal and basolateral surfaces of epithelial cells, and exists across many species, including humans, cattle, pigs, dogs, mice, rats, and poultry (Lax *et al.,* 1988; Playford and Wright, 1996). There is a body of literature demonstrating gut health and function benefits of supplemental exogenous EGF in neonate or weaned mammals, however, its ability to stimulate gastrointestinal growth and function in broiler chicks has not been thoroughly assessed (Barnard *et al.,* 1995; Kang *et al.,* 2010). Therefore, the objective of this study was to evaluate the effects of EGF supplementation on growth performance, nutrient retention, indices of gut health and function in broilers subjected to *Eimeria* challenge. It

was hypothesized that supplying exogenous EGF would promote gastrointestinal development in chicks and reduce the negative effects of *Eimeria* challenge, in regards to growth performance, and indices of gut health and function.

Materials and Methods

The experimental protocol was reviewed and approved by the University of Guelph Animal Care Committee. Birds were cared for in accordance with the Canadian Council on Animal Care guidelines (CCAC, 2009).

Birds and housing

A total of 216 male day old broiler chicks were procured from a local hatchery (Maple Leaf Foods, New Hamburg, ON, Canada). Prior to delivery, the hatchery was instructed not to vaccinate birds with the cocci-vaccine. The chicks were housed in an environmentally controlled room at Arkell Poultry Research Station, Guelph ON, Canada. The room contained a total of 64 cages installed in two rows, separated by a 36" walkway and with cages of 16 in a row, stacked in two tiers. The birds were allocated to 36 cages (6 birds per cage) based on day 0 body weights. Room temperature and lighting program, in addition to care and handling to birds followed Arkell Research Station SOP.

Experimental treatments and feeding

A basal diet was formulated to meet breeder (Ross 708) nutrient specifications (Table 1). The diet was prepared in mash form, containing TiO_2 as the indigestible marker, and had no antibiotics or anti-coccidial drugs. Three experimental treatments were tested: 1) control (fermentation supernatant without EGF), 2) EGF containing supernatant (80 μg/kg of BW/d), and 3) EGF containing supernatant (160 μg/kg of BW/d). The EGF was supplied fresh on a daily basis in accord to the previous piglet trials (Kang *et al.*, 2010; Bedford *et al.*, 2012; Bedford *et al.*, 2015). The absolute amount of EGF allocated to each pen was determined daily, based upon the number of chicks per cage and expected breeder's growth curve (Aviagen, Ross 708). The EGF chicks were given volumes of supernatant to achieve 80 and 160 μg/kg of BW/d throughout the study, while the control chicks were given 80 μg of fermentation supernatant/kg of BW/d.

Table 1. Ingredients composition of the basal diet (*%, as fe*d)

Ingredient, %	Amount %
Corn	38.54
Soybean meal	37.21
Rye	10.00
Animal-Veg Fat	8.70
Poultry Vit/Min Premix	1.00
Mono calcium phosphate	1.50
Limestone	1.33
Sodium bicarbonate	0.51
Titanium dioxide	0.50
DL-Methionine	0.32
L-Lysine-HCL	0.14
L-Threonine	0.10
Tryptophan	0.10
Salt	0.04
Titanium dioxide	0.50
Calculated provisions	
AME, mcal/kg	3.10
CP, %	21.50
SID Lys, %	1.15
SID Met, %	0.60
SID Met + Cys, %	0.87
SID Thr, %	0.77
SID Trp, %	0.33
Ca, %	0.87
P, %	0.65
Avail. P, %	0.44
Na, %	0.16

[1]Vitamin mineral premix provided per kilogram of diet: vitamin A, 880,000 IU; vitamin D3, 330,000 IU; vitamin E, 4,000 IU; vitamin B12, 1200 mcg; biotin, 22000 mcg; menadione, 330 mg; thiamine, 400 mg; riboflavin, 800 mg; pantothenic acid, 1500 mg; pyridoxine, 300 mg; niacin, 5,000 mg; folic acid, 100 mg; choline, 60,000 mg; iron, 6,000 mg; copper, 1000 mg

Experimental procedures and sampling

The three treatments were allocated to cages through completely randomized block (row of cages) design to give 12 replicates per treatment. Birds had free access to feed and drinking water for 14 d; feed was replenished through the day and feed refusals were weighed daily for determining feed intake. On d 5, 108 birds (6 replicates per treatment, left rows of cages) were challenged with 1 mL of *Eimeria* culture (25,000 oocysts of *E. acervulina* and 5,000 oocysts of *E. maxima*) via oral gavage and the other 6 replicates (non-challenged control, right rows of cages) were given equal volumes of distilled water. The separation of right and left cages was an effort to minimize cross-contamination of the non-challenge cages and daily checks of the birds started with non-challenged followed by the challenged birds. The *Eimeria* culture and challenge protocols were provided by Dr. John Barta of the Department of Pathobiology, University of Guelph. Body weight and feed intake was monitored during pre- (d 0-5) and post- (d 6-14) challenge periods for calculation of BWG and FCR.

Two birds per cage were sacrificed on d 10 for intestinal tissue samples and intestinal lesion scores. Segments of (~3 cm) of mid-jejunum were excised and placed in buffered formalin for histomorphology analysis (Kiarie *et al.*, 2007). Additional (~1 cm) segments of mid-jejunum were

excised and placed in a 2 mL tube filled with 1.2 mL Ambion® RNAlater (Life Technologies Inc., Burlington, ON, Canada). These samples were placed on ice, transported back to the lab and stored at -20°C until required for mRNA analysis of digestive enzymes (maltase and sucrase), nutrients transporters; cationic AA transporter (CAT1), and sodium glucose transporter 1 (SGLT1), tight junction proteins such as occuludin (OCLN), proliferating cell nuclear antigen (PCNA), and cytokines (TLR2 and TLR4). Lesion scores were assessed blindly in intestinal regions (duodenum, jejunum, ileum, and ceca) as described by Price *et al.* (2014) using a scale of 0 (none) to 4 (high) (Johnson and Reid, 1970). Excreta samples for apparent retention (AR) of components and oocyst shedding were collected and stored at -20ºC and 4ºC respectively until required for analysis. All birds were killed on d 14 for gastrointestinal weight measurements.

Calculations and Statistical Analysis

The AR of components were calculated according to (Kiarie *et al.*, 2014). Pre-challenge growth performance data was subjected to 1-way ANOVA of the GLM procedures (SAS Inst. Inc., Cary, NC), with diet as a fixed factor. Post-challenge growth performance (with d5 BW as co-variate), gastrointestinal measurements and AR of components data were subjected to 2-way ANOVA with fixed effects of treatment, *Eimeria* challenge, and their interactions. Oocyst shedding in challenged birds were analyzed using the Proc Mixed procedure of SAS as repeated measurements with the fixed effects of treatment, time, and treatment X time interaction. Similarly, lesion scores for the challenged birds was subjected to 1-way ANOC with treatment as fixed effect. Linear and quadratic responses of EGF were also assessed. An α level of $P \leq 0.05$ was used as the criterion for statistical significance.

Results and Discussion

Management of perinatal nutrition of a chick is identified as a major influence for the growth and efficiency of the modern broiler (Uni and Ferket, 2004; Ferket, 2012). Early access to nutrients and factors not only promotes greater growth and feed efficiency, but also favours the development of the immune system, allowing the bird to build a greater resistance to pathogenic organisms (Mitchell and Smith, 1991; Croom *et al.*, 1999; Sklan *et al.*, 2003; Gilbert *et al.*, 2007). Epidermal growth factor (EGF) is a heat and acid stable peptide that produces a variety of biological responses, many of which are involved in the regulation of cell replication, cell movement, and cell survival (Playford and Wright, 1996; Oda *et al.*, 2005). In the gastrointestinal tract, EGF plays a role in the proliferation and differentiation of epithelial cells, however, EGF also has significant effects on the healing of damaged mucosa or the intestinal adaptation after injury (James *et al.*, 1987; Barnard *et al.*, 1995; Kang *et al.*, 2010).

Growth performance and nutrient retention

EGF linearly (P=0.03) increased BWG before challenge, however, there was no ($P > 0.05$) effect of EGF on feed intake and FCR before challenge (Table 2). Although the effects of EGF on gut development was not assessed on d 5, the observed improvement of BWG on d 5 could be linked to the positive effects of EGF on gut development. However, when growth performance data for

the non-challenged birds was examined from d 0 to 14, there was no effect of EGF on BWG, feed intake, and FCR (Table 2), suggesting the possibility of a EGF dosage issue. Growth performance data for the post-challenge period (d 6-14) is shown in Table 3. There was no ($P > 0.05$) interaction between *Eimeria* challenge and treatment or the main effects of EGF. The main effect ($P < 0.01$) of *Eimeria* challenge depressed BWG and feed intake, while FCR increased. The main effects of *Eimeria* challenge were such that *Eimeria* depressed ($P < 0.01$) AR of DM, fat, and gross energy (Figure 1).

Table 2. Growth performance of broiler chicken fed corn diet without or with epidermal growth factors, pre-challenge

EGF[1], µg	Initial body weight, g	Day, 0-5			Day, 0-14		
		Body weight gain, g	Feed intake, g	FCR	Body weight gain, g	Feed intake, g	FCR
0	40.8	45.7b	82.8	1.813	320	675	2.155
80	41.0	48.6a	84.1	1.738	331	678	2.094
160	41.2	49.9a	85.6	1.719	356	655	1.840
SEM	0.36	1.00	1.61	0.054	15.56	21.80	0.145
P- value	-	0.029[L]	0.484	0.237	0.121	0.715	0.147

[1]Epidermal growth factor, µg/kg body of chick based on projected growth curve of Ross 708.
Means assigned different letters within a response criteria are significantly different, $P < 0.05$.
[L]linear effects of EGF.

Table 3. Growth performance in broiler chickens fed corn-soybean meal based diets without or with epidermal growth factor and challenged with *Eimeria*

Eimeria[1]	EGF[2], µg	Final body weight, g	Body weight gain, g	Feed intake, g	FCR
No	0	373	284	589	2.113
No	80	372	282	594	2.171
No	160	390	301	570	1.922
Yes	0	264	174	460	2.699
Yes	80	277	186	469	2.813
Yes	160	265	176	462	2.640
	SEM	15.3	15.3	23.3	0.270
Probabilities					
Eimeria		<0.01	<0.01	<0.01	0.006
EGF		0.836	0.844	0.810	0.744
*Eimeria**EGF		0.619	0.630	0.892	0.971

1Chicks were orally gavaged with a 1 mL mixture of 25,000 E. acervulina and 5,000 E. maxima on d 5.
2Epidermal growth factor, µg/kg BW based on projected growth curve of Ross 708. Means assigned different letters within a factor of analysis (*Eimeria*, EGF and their interactions) are significantly different, $P < 0.05$.

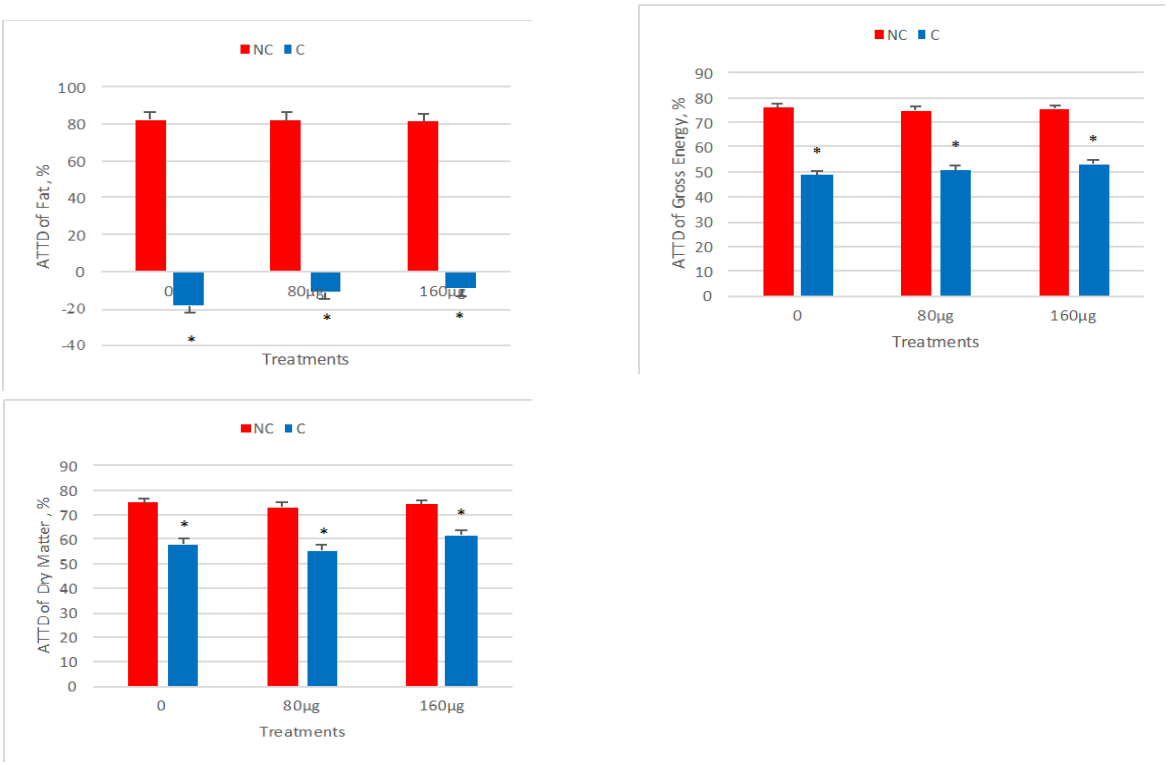


Figure 1. Apparent retention (AR) of DM, fat, and gross energy in broiler chickens fed corn-soybean meal based diets with or without epidermal growth factor and challenged with *Eimeria* (with non-challenged represented by the notation "NC" and challenged represented by "C")

Oocyst shedding, lesion scores, gut weight, and histomorphology

For poultry, different species of *Eimeria* are known to develop in different regions of the gut and, depending on the magnitude of infection, can cause mild to severe lesions and significant pathology including death (Chapman, 2014; Chapman *et al.*, 2016). Extending from the duodenum to the mid-intestine, *E. acervulina and E. praecox* infection occurs, E. mitis, E. maxima, and E. necatrix develops in the mid-intestine extending to the posterior intestine, and E. tenella develops in the ceca (Joyner *et al.*,1978). The hallmark of coccidiosis is the fluid loss and malabsorption of nutrients, inflammation of the intestinal wall, pinpoint hemorrhages, sloughing of epithelia, or complete villi destruction, resulting in extensive hemorrhage (Chapman, 2014; Chapman *et al.*, 2016). These distinct characteristics have been widely accepted and used to create a visual system for scoring the severity of lesion in different regions of the gut (Johnson and Reid, 1970).

With this said, oocyst shedding and intestinal (duodenum, jejunal, and ileum) lesion scores were only observed in birds challenged with *Eimeria* and there was no EGF effects ($P > 0.05$) on these parameters (data not shown). The oocyst shedding was 87,479, 910,449, 428583 and 57,868 (SEM=48,074) oocysts per gram of excreta for d 5, 6, 7, and 8 post-challenge, respectively. The duodenal lesion scores 2.33, 2.50 and 2.75 (SEM = 0.26) for the control, 80 µg of EGF/kg of BW/d and 160 µg of EGF/kg BW/d, respectively; the respective values for the jejunum were 1.55, 1.55 and 1.67 (SEM = 0.21) and ileum 0.35, 0.42 and 0.33 (SEM = 0.18). However, the lack of EGF effects on lesion scores and oocyst shedding suggests negligible effects on attenuating *Eimeria* infection of the intestines.

The main effects of *Eimeria* challenge were such that challenged birds exhibited heavier ($P < 0.01$) gizzard, small intestine, and ceca weights than that of non-challenged control birds (Figure 2). *Eimeria* challenge reduced ($P < 0.01$) villi height and increased ($P < 0.01$) crypt depth, resulting in decreased villi to crypt depth ratio ($P < 0.01$) (data not shown). Comprehensive review of avian gastrointestinal development shows that the small intestine of a hatchling accounts for a larger percentage of whole body weight peaking at d 7-10 post-hatch (Wijtten *et al.*, 2012). For the present study, taking the gastrointestinal tract measurements at an earlier age would then have been ideal for the assessment of EGF effects on gastrointestinal development.

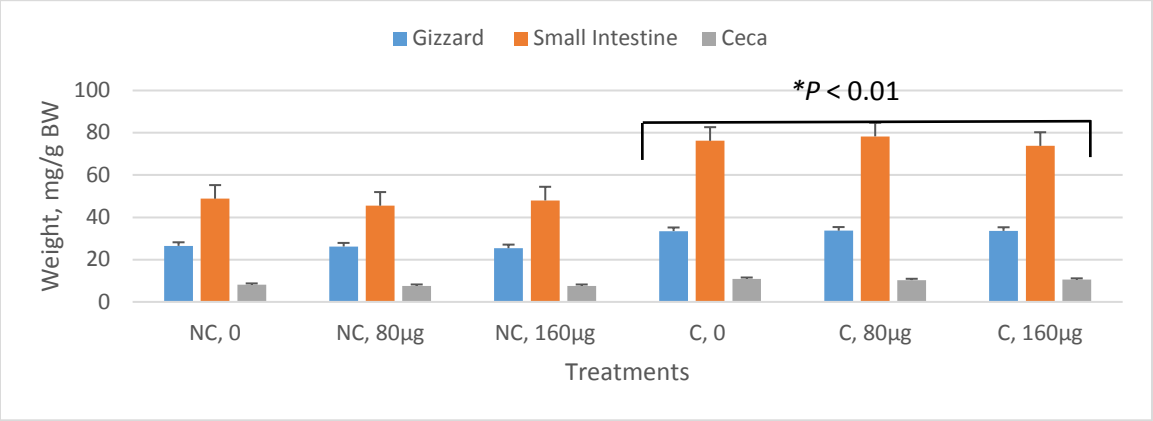


Figure 2. Relative gastrointestinal weight of broiler chicks fed corn-soybean meal based diets with or without epidermal growth factor and challenged with *Eimeria* (with non-challenged represented by the notation "NC", challenged represented by "C", and * being an *Eimeria* effect)

Jejunal expression of genes for digestive enzymes, nutrient transporters, cytokines, and tight junction proteins

Although we did not observe effects of EGF on jejunal histomorphology, improved expression of nutrient transporters (CAT1 and SGLT1) and digestive enzymes (sucrase and maltase) was seen. An interaction between *Eimeria* challenge and EGF expression of maltase and sucrase was observed such that EGF improved expression of maltase (linear, $P = 0.02$) and sucrase (quadratic, $P = 0.04$) in only the *Eimeria* challenged birds. The data for nutrient transporters is shown in Table 4. Interaction between *Eimeria* and EGF was observed for expression of CAT1 and SGLT1. In this context, birds fed EGF maintained similar ($P > 0.05$) expression of SGLT1, without or with *Eimeria* challenge whilst expression of these genes was downregulated ($P < 0.05$) by *Eimeria* challenge in control birds. With respect to CAT1, EGF increased expression linearly. These observations extended our previous observations in piglets, in which feeding EGF supernatant, increased the expression of SGLT1, and glucagon-like peptide-2, mucin-2, and goblet cells (Bedford *et al.*, 2015). Collectively suggesting that EGF could play a role in attenuating some of the negative effects of *Eimeria* invasion on chicken digestive and absorptive capacity.

Toll-like receptors (TLRs) are important components of the avian gut innate immune system that sense conserved microbial patterns and endogenous danger signals while tight junction proteins are important for maintaining the barrier property of a tight junction (Abreu *et al.*, 2005; Satoh and Akira, 2016). An interaction between *Eimeria* challenge and EGF was seen on expression of TLR4 and tight junction protein OCLN such that EGF linearly increased expression of these genes in only the challenged birds (Table 5). The expression of OCLN in challenged birds was greatest in

conjunction with the highest dose of EGF compared to non-challenged birds receiving this dose. Increased expressions of these genes suggests that EGF may have had a protective role in the gastrointestinal tract against colonization of *Eimeria.*

Cell proliferation in the intestine commonly occurs during infection and inflammation to replace damaged enterocytes (Buret *et al*., 2002; Buret *et al*., 2003; Lamb-Rosteski *et al*., 2008). Proliferating cell nuclear antigen (PCNA) is a cofactor of DNA polymerase δ that is highly expressed at the S-phase of the cell cycle and thus, has been used as a marker of cell proliferation (Muskhelishvili et al., 2003). The interaction ($P = 0.05$) between *Eimeria* challenged and EGF on PCNA was such that challenged birds receiving the highest dose of EGF had higher expression of PCNA than in non-challenged birds receiving the same dose, suggesting that EGF may have had potential therapeutic benefits for the *Eimeria* challenged birds.

Eimeria invasion of the intestinal epithelium gives rise to weight loss, poor feed conversion, diarrhea, and in extreme cases, death (Major and Ruff, 1978). *Eimeria* challenge resulted in structural and functional damages in the small intestine as demonstrated by the histomorphology, digestive enzymes, nutrient transporters and nutrient retention data. The current study demonstrated that *Eimeria* challenge reduced growth performance and impaired gut function as expected. However, EGF also showed beneficial effects on pre-challenge growth and improved indices of gut function upon *Eimeria* challenge, highlighting its potential therapeutic benefits and protective role of the gastrointestinal tract from colonization of pathogens and from induced barrier defects.

Table 4. Jejunal expression of genes for nutrient transporters and digestive enzymes in broiler chickens fed corn-soybean meal based diets without or with epidermal growth factor and challenged with *Eimeria*

Treatments		Nutrient transporters[3]		Enzymes	
Eimeria[1]	EGF[2], µg	CatAA	SGLT1	Maltase	Sucrase
No	0	0.29b	59.3a	4.59ab	26.2ab
No	80	0.44b	48.4ab	3.88ab	17.3ab
No	160	0.41b	50.7ab	2.12b	16.9b
Yes	0	0.49b	32.8b	2.18b	18.3ab
Yes	80	0.80ab	40.2ab	4.32ab	35.8a
Yes	160	1.26a	57.0a	6.15a	33.5ab
	SEM	0.23	8.41	1.07	6.37
Probabilities					
Eimeria		0.020	0.177	0.437	0.094
EGF		0.170	0.493	0.736	0.785
*Eimeria**EGF		0.009^{L}	0.034	0.020^{L}	0.039^{Q}

[1]Chicks were orally gavaged with a 1 mL mixture of 25,000 *E. acervulina*, 5,000 *E. maxima* on d 5.
[2]Epidermal growth factor, µg/kg body of chick based on projected growth curve of Ross 708
[3]NAA, neutral amino acids transporter; Glut, glutamate transporter; CatAA, cationic amino acids transporter; SGLT1, sodium dependent glucose transporter; PepT1, Peptide transporter 1;1xCT1, cysteine-glutamate exchanger
Means assigned different letters within a factor of analysis (*Eimeria*, EGF and their interactions) are significantly different, $P < 0.05$.
[L]linear and [Q]quadratic effects of EGF.

Table 5. Jejunal expression of genes for cytokines, tight junction proteins and proliferating cell nuclear antigen in broiler chickens fed corn-soybean meal based diets without or with epidermal growth factor and challenged with *Eimeria*

Treatments		Toll like receptors[3]	Tight junction proteins[4]	PCNA[5]
Eimeria[1]	EGF[2], µg	TLR4	Occuludin	
No	0	0.23ab	1.21ab	3.69ab
No	80	0.36ab	0.73ab	3.55ab
No	160	0.40ab	0.56b	3.47b
Yes	0	0.12b	0.64b	3.57ab
Yes	80	0.43ab	0.93ab	5.81a
Yes	160	0.84a	1.29a	5.88a
	SEM	0.22	0.22	0.81
Probabilities				
Eimeria		0.472	0.504	0.031
EGF		0.054	0.878	0.335
*Eimeria**EGF		0.026[L]	0.021[L]	0.048

[1]Chicks were orally gavaged with a 1 mL mixture of 25,000 E. acervulina, 5,000 E. maxima on day 5, intestinal samples collected 5 (day 10 of life) days after challenge.
[2]Epidermal growth factor, µg/kg body of chick based on projected growth curve of Ross 708.
[3]Toll-like receptor 4.
[4]Occludin
[5]Proliferating Cell Nuclear Antigen.
Means assigned different letters within a factor of analysis (*Eimeria*, EGF and their interactions) are significantly different, $P < 0.05$.
[L]Linear effects of EGF.

References

Abreu, M. T., M. Fukata, and M. Arditi. 2005. TLR signaling in the gut in health and disease. Journal of Immunology 174: 4453-4460.

Akira, S., and K. Takeda. 2004. Functions of Toll-like receptors: lessons from KO mice. Cr Biol 327: 581-589.

Barnard, J. A., R. D. Beauchamp, W. E. Russell, R. N. Dubois, and R. J. Coffey. 1995. Epidermal growth factor-related peptides and their relevance to gastrointestinal pathophysiology. Gastroenterology 108: 564-580.

Bedford, A., T. Chen, E. Huynh, C. Zhu, S. Medeiros, D. Wey, C. de Lange, and J. Li. 2015. Epidermal growth factor containing culture supernatant enhances intestine development of early-weaned pigs in vivo: potential mechanisms involved. J Biotechnol 196-197: 9-19.

Bedford, A., Z. Li, M. Li, S. Ji, W. Liu, Y. Huai, C. F. de Lange, and J. Li. 2012. Epidermal growth factor-expressing Lactococcus lactis enhances growth performance of early-weaned pigs fed diets devoid of blood plasma. J Anim Sci 90 Suppl 4: 4-6.

Buret, A., M. E. Olson, D. G. Gall, and J. A. Hardin. 1998. Effects of orally administered epidermal growth factor on enteropathogenic Escherichia coli infection in rabbits. Infect Immun 66: 4917-4923.

Buret, A. G., A. C. Chin, and K. G. Scott. 2003. Infection of human and bovine epithelial cells with Cryptosporidium andersoni induces apoptosis and disrupts tight junctional ZO-1: effects of epidermal growth factor. Int J Parasitol 33: 1363-1371.

Buret, A. G., K. Mitchell, D. G. Muench, and K. G. Scott. 2002. Giardia lamblia disrupts tight junctional ZO-1 and increases permeability in non-transformed human small intestinal epithelial monolayers: effects of epidermal growth factor. Parasitology 125: 11-19.

CCAC. 2009. Guidelines on: The care and use of farm animals in research, teaching and testing. In: C. C. o. A. Care (ed.), Ottawa, On, Canada.

Chapman, H. D. 2009. A landmark contribution to poultry science--prophylactic control of coccidiosis in poultry. Poult Sci 88: 813-815.

Chapman, H. D. 2014. Milestones in avian coccidiosis research: A review. Poultry Sci 93: 501-511.

Chapman, H. D., J. R. Barta, M. A. Hafeez, P. Matsler, T. Rathinam, and M. Raccoursier. 2016. The epizootiology of Eimeria infections in commercial broiler chickens where anticoccidial drug programs were employed in six successive flocks to control coccidiosis. Poult Sci.

Croom, W. J., J. Brake, B. A. Coles, G. B. Havenstein, V. L. Christensen, B. W. McBride, E. D. Peebles, and I. L. Taylor. 1999. Is intestinal absorption capacity rate-limiting for performance in poultry? J Appl Poultry Res 8: 242-252.

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

Gilbert, E. R., H. Li, D. A. Ernmersonj, K. E. Webb, and E. A. Wong. 2007. Developmental regulation of nutrient transporter and enzyme mRNA abundance in the small intestine of broilers. Poultry Sci 86: 1739-1753.

Jaeger, L. A., C. H. Lamar, T. R. Cline, and C. J. Cardona. 1990. Effect of Orally-Administered Epidermal Growth-Factor on the Jejunal Mucosa of Weaned Pigs. Am J Vet Res 51: 471-474.

James, P. S., M. W. Smith, D. R. Tivey, and T. J. G. Wilson. 1987. Epidermal Growth-Factor Selectively Increases Maltase and Sucrase Activities in Neonatal Piglet Intestine. J Physiol-London 393: 583-594.

Johnson, J., and W. M. Reid. 1970. Anticoccidial drugs: lesion scoring techniques in battery and floor-pen experiments with chickens. Exp Parasitol 28: 30-36.

Joyner, L. P., E. U. Canning, P. L. Long, D. Rollinson, and R. B. Williams. 1978. A suggested terminology for populations of coccidia (Eimeriorina), particularly of the genus Eimeria (Protozoa: Apicomplexa). Parasitology 77: 27-31.

Kang, P., D. Toms, Y. L. Yin, Q. Cheung, J. Gong, K. De Lange, and J. L. Li. 2010. Epidermal Growth Factor-Expressing Lactococcus lactis Enhances Intestinal Development of Early-Weaned Pigs. J Nutr 140: 806-811.

Kiarie, E., C. M. Nyachoti, B. A. Slominski, and G. Blank. 2007. Growth performance, gastrointestinal microbial activity, and nutrient digestibility in early-weaned pigs fed diets containing flaxseed and carbohydrase enzyme. J Anim Sci 85: 2982-2993.

Kiarie, E., L. F. Romero, and V. Ravindran. 2014. Growth performance, nutrient utilization, and digesta characteristics in broiler chickens fed corn or wheat diets without or with supplemental xylanase. Poult Sci 93: 1186-1196.

Lamb-Rosteski, J. M., L. D. Kalischuk, G. D. Inglis, and A. G. Buret. 2008. Epidermal growth factor inhibits Campylobacter jejuni-induced claudin-4 disruption, loss of epithelial barrier function, and Escherichia coli translocation. Infect Immun 76: 3390-3398.

Lax, I., A. Johnson, R. Howk, J. Sap, F. Bellot, M. Winkler, A. Ullrich, B. Vennstrom, J. Schlessinger, and D. Givol. 1988. Chicken epidermal growth factor (EGF) receptor:

cDNA cloning, expression in mouse cells, and differential binding of EGF and transforming growth factor alpha. Mol Cell Biol 8: 1970-1978.

Lilja, C. 1983. A Comparative-Study of Postnatal-Growth and Organ Development in Some Species of Birds. Growth 47: 317-339.

Major, J. R., Jr., and M. D. Ruff. 1978. Eimeria spp.: influence of coccidia on digestion (amylolytic activity) in broiler chickens. Exp Parasitol 45: 234-240.

Mitchell, M. A., and M. W. Smith. 1991. The effects of genetic selection for increased growth rate on mucosal and muscle weights in the different regions of the small intestine of the domestic fowl (Gallus domesticus). Comp Biochem Physiol A Comp Physiol 99: 251-258.

Muskhelishvili, L., J. R. Latendresse, R. L. Kodell, and E. B. Henderson. 2003. Evaluation of cell proliferation in rat tissues with BrdU, PCNA, Ki-67(MIB-5) immunohistochemistry and in situ hybridization for histone mRNA. J Histochem Cytochem 51: 1681-1688.

Oda, K., Y. Matsuoka, A. Funahashi, and H. Kitano. 2005. A comprehensive pathway map of epidermal growth factor receptor signaling. Mol Syst Biol 1: 2005 0010.

Playford, R. J., and N. A. Wright. 1996. Why is epidermal growth factor present in the gut lumen? Gut 38: 303-305.

Price, K. R., M. T. Guerin, and J. R. Barta. 2014. Success and failure: The role of relative humidity levels and environmental management in live Eimeria vaccination of cage-reared replacement layer pullets. J Appl Poultry Res 23: 523-535.

Roberts, T., J. Wilson, A. Guthrie, K. Cookson, D. Vancraeynest, J. Schaeffer, R. Moody, and S. Clark. 2015. New issues and science in broiler chicken intestinal health: intestinal microbial composition, shifts, and impacts. World Poultry Sci J 71: 259-269.

Satoh, T., and S. Akira. 2016. Toll-Like Receptor Signaling and Its Inducible Proteins. Microbiol. Spectr. 4.

Singh, A. B., and R. C. Harris. 2004. Epidermal growth factor receptor activation differentially regulates claudin expression and enhances transepithelial resistance in Madin-Darby canine kidney cells. J Biol Chem 279: 3543-3552.

Sklan, D., S. Heifetz, and O. Halevy. 2003. Heavier chicks at hatch improves marketing body weight by enhancing skeletal muscle growth. Poultry Sci 82: 1778-1786.

Su, S., K. B. Miska, R. H. Fetterer, M. C. Jenkins, and E. A. Wong. 2014. Expression of digestive enzymes and nutrient transporters in Eimeria acervulina-challenged layers and broilers. Poult Sci 93: 1217-1226.

Sun, L., H. Dong, Z. Zhang, J. Liu, Y. Hu, Y. Ni, R. Grossmann, and R. Zhao. 2016. Activation of epithelial proliferation induced by Eimeria acervulina infection in the duodenum may be associated with cholesterol metabolism. Oncotarget 7: 27627-27640.

Uni, Z., and R. P. Ferket. 2004. Methods for early nutrition and their potential. World Poultry Sci J 60: 101-111.

Uni, Z., A. Geyra, H. Ben-Hur, and D. Sklan. 2000. Small intestinal development in the young chick:crypt formation and enterocyte proliferation and migration. Br Poult Sci 41: 544-551.

Uni, Z., Y. Noy, and D. Sklan. 1999. Posthatch development of small intestinal function in the poult. Poultry Sci 78: 215-222.

Uni, Z., R. Platin, and D. Sklan. 1998. Cell proliferation in chicken intestinal epithelium occurs both in the crypt and along the villus. J Comp Physiol B 168: 241-247.

Wijtten, P. J. A., D. J. Langhout, and M. W. A. Verstegen. 2012. Small intestine development in chicks after hatch and in pigs around the time of weaning and its relation with nutrition: A review. Acta Agriculturae Scandinavica, Section A - Animal Science 62: 1-12.

Exploring Modes of Action and Efficacy for Alternative Gut Health Modulating Additives in Young Pigs

Examen des modes d'action et de l'efficacité des additifs de rechange de modulation de la santé intestinale chez les jeunes porcs

John R. Pluske[1]
[1]School of Veterinary and Life Sciences, Murdoch University, MURDOCH, WA 6150, AUSTRALIA; J.Pluske@murdoch.edu.au

Sponsor / *Commanditaire*:

Abstract

New and (or) different management and feeding strategies are needed to appropriately manage the gastrointestinal tract (GIT) in the peri-weaning period in order to maintain, and indeed improve, current production indices whilst minimizing the use of some antimicrobial compounds. Restrictions and bans on the use of some antimicrobial compounds, mainly antibiotics, coupled with changing perceptions and attitudes have consequently resulted in a marked increase in both the interest and use of alternative feed additives for the young pig. However the complexity of the GIT and its interactions with the host makes universal recommendations very challenging. In this context, it is important to distinguish between alternative feed additives for disease prevention, for growth promotion, and those for disease treatment (e.g., prophylaxis *vs.* therapy), as they will generally be different in accordance with mechanisms of action. Combinations of antibiotic alternatives also hold the promise of potentiating each other's efficacy and duplicating the effects of in-feed antibiotics. The array of potential alternative feed additives in the marketplace is large and growing, unfortunately inconsistent results under both experimental and field (practical) conditions hamper comprehensive advances and recommendations. Nevertheless, new research tools such as metagenomics and other genome-enabled technologies are providing fresh insights into mechanisms pertaining to ecology of the microbiome, host–pathogen interactions, immune development and nutrition – health interactions, which provide new opportunities for developing alternative strategies to enhance the production and health of young pigs.

Résumé

De nouvelles et (ou) différentes stratégies de gestion et d'alimentation sont nécessaires pour gérer correctement le tube digestif durant la période entourant le sevrage afin de maintenir (voire d'améliorer) les indices de production actuels tout en diminuant au maximum l'usage de certains composés antimicrobiens. Les restrictions et interdictions visant l'utilisation de certains composés antimicrobiens jumelées à l'évolution des perceptions et attitudes des consommateurs

et détaillants ont entraîné une nette augmentation de l'intérêt pour la production et l'utilisation d'additifs alimentaires de substitution chez les porcelets, bien que la complexité du tube digestif et de ses interactions avec l'hôte rende très difficile la formulation de recommandations universelles. Dans ce contexte, il est important de faire la distinction entre les additifs alimentaires de substitution utilisés pour la prévention des maladies et la stimulation de la croissance et ceux servis pour le traitement des maladies (c'est-à-dire, prophylaxie vs thérapie), puisque leurs modes d'action seront généralement différents. Par exemple, si leur mode d'action dépend du microbiote ou de son interaction avec le système immunitaire, les additifs alimentaires comme les prébiotiques / probiotiques, les produits phytogènes ou les immuno-modulateurs sont probablement appropriés. Par contre, si leur mécanisme de stimulation de la croissance repose sur la prévention et la réduction de la maladie, les meilleures solutions sont probablement des vaccins ou des prébiotiques / probiotiques de promotion de la santé. Des combinaisons de produits antibiotiques de remplacement offrent aussi la possibilité de potentialiser l'activité de chacun des composants et de reproduire l'effet des antibiotiques utilisés dans les aliments. Il existe une panoplie d'additifs alimentaires de substitution potentiels sur le marché et leur nombre augmente, mais malheureusement les résultats variables obtenus en conditions expérimentales et au champ (en conditions pratiques) gênent la recherche. Quoi qu'il en soit, de nouveaux outils scientifiques, tels que la métagénomique et d'autres technologies fondées sur le génome, permettent d'acquérir de nouvelles connaissances sur l'écologie du microbiome, sur les interactions entre les agents pathogènes et leur hôte, sur le développement de l'immunité et sur les interactions entre la nutrition et la santé, ce qui pave la voie à l'élaboration d'autres stratégies d'amélioration de la production et de la santé des porcelets.

Introduction

There is wide interest in developing and implementing management and feeding strategies to maintain/increase current production levels after weaning in a regulatory and (or) external environment where certain antimicrobial agents, such as antibiotics, are not permitted for use. In Canada, as in some other parts of the world, the evolving landscape with regard to the use of antibiotics (medically important antibiotics) will bring challenges associated with the post-weaning transition and cause the experimentation and adoption of alternative feeding/water application strategies. In a Health Canada news release published June 30, 2016 (http://news.gc.ca/web/article-en.do?nid=1092499), a Federal Action Plan on Antimicrobial Resistance was outlined that identifies steps the government will undertake in the areas of (1) *Surveillance* (detecting and monitoring trends and threats to inform strategies to reduce the risks and impacts of antimicrobial resistance), (2) *Stewardship* (conserving the effectiveness of existing treatments through infection prevention and control guidelines, education and awareness, regulations, and oversight), and (3) *Innovation* (finding new solutions to counteract loss in antimicrobial effectiveness through research and development), “in protecting Canadians against the increasing risk of antimicrobial resistance”. Of particular interest is the future of the use of antibiotic growth promoters (AGPs), which are used routinely in some production systems to assist the young pig in the weaning transition.

Antibiotics have been administered to pigs, and indeed other agricultural animals, for in excess of 60 years for disease treatment, disease prevention, and growth promotion (e.g., Anderson et al., 1999; Dibner and Richards, 2005; Looft et al., 2012). Restrictions and bans on use will by necessity cause new and (or) different management and feeding strategies to be introduced to appropriately manage the gastrointestinal tract (GIT) in the peri-weaning period in order to maintain/improve current production measures. There is no shortage in the marketplace of feed

and (or) water additives available to pork producers to use as alternatives or replacements, and a large number of feed additives have been evaluated that are generally aimed at either (1) enhancing the pigs' immune responses (e.g., immunoglobulin; ω-3 fatty acids, yeast derived ß-glucans), (2) reducing pathogen load in the pig's GIT (e.g., organic and inorganic acids, high levels of zinc oxide, essential oils, herbs and spices, some types of prebiotics, bacteriophages, anti-microbial peptides), (3) stimulating establishment of beneficial GIT microbes (e.g., probiotics and some types of prebiotics), and (4) stimulating digestive function (e.g., butyric acid, gluconic acid, lactic acid, glutamine, threonine, cysteine, and nucleotides) (de Lange et al., 2010). Nevertheless, the widespread use of some of these products in the pork industry is limited, and indeed questioned, by the lack of confirmation pertaining to their efficacy, reliability and (or) mode(s) of action. A likely reason for this inconsistency is the wide range of production conditions under which the additives are expected to provide a positive response, commensurate with the lack of a full understanding of their explicit mode(s) of action. Examination of the modes of action of antibiotics may provide some insights in this respect.

The purpose of this review is to first examine the purported modes of action of antibiotics, as a basis for trying to understand how changes to the structure and function of the GIT are modified, and second to outline broadly the mode(s) of action of a number of additives available as replacements for antibiotics. There is a plethora of studies already published, too numerous to mention, related to this topic, hence it is beyond this contribution to summarize all this information. The focus of this brief review is to highlight key underlying concepts that allow a better understanding of the value of selected feed additives to stimulate GIT "health" (structure and function) and development in the newly-weaned pig, although clearly concepts may be relevant to older pigs also.

How do Antibiotics Work?

Dibner and Richards (2005) commented that the mechanism of action of antibiotics, particularly AGPs, must be focused on the GIT because some of these antibiotics are not absorbed. Early studies indicated that oral antibiotics do not have growth-promoting effects in germ-free animals (Coates et al., 1955; Coates et al., 1963), surmising that the mechanism for growth promotion should be focused on interactions between the antibiotic and the GIT bacteria (microbiota). Thus, direct effects of antibiotics on the GIT bacteria can be used to explain decreased competition for nutrients and reduction in microbial metabolites that depress growth (Visek, 1978; Anderson et al., 1999). Additional antibiotic effects that also occur in germ-free animals include the reduction in GIT size, comprising thinner intestinal villi and the total GIT wall (Coates et al., 1955). Frankel et al. (1994) commented that this may be due, at least in part in part, to the loss of mucosal cell proliferation in the absence of short-chain fatty acids derived from microbial fermentation. The reduction in GIT wall thickness and the villous lamina propria has been used to explain the enhanced nutrient digestibility observed with AGP (see Dibner and Richards, 2005). This led Gaskins et al. (2002) to comment that antibiotics improve the efficiency of animal growth via their inhibition of the normal microbiota, leading to increased nutrient utilization and a reduction in the maintenance costs of the GIT system. Collier et al. (2003) remarked that AGPs may also improve pig performance, in part, by decreasing bacterial colonization of the small intestine, supporting the concept that antibiotic alternatives might ideally promote the growth of beneficial commensal bacteria, while suppressing those that are deleterious. A reduction in opportunistic pathogens and subclinical infection has also been linked to use of AGP. Injection of bacterial metabolites such as lipopolysaccharides or immune mediators such as interleukin-1 can mimic the reduced

efficiency of an animal with a conventional microbiota and no antimicrobial in the diet (Roura et al., 1992), which further illustrates the importance of the host response to the microbiota as an integral factor limiting growth efficiency. Moreover, Lin (2014) stated that the growth-promoting effect of AGPs was correlated with the decreased activity of bile salt hydrolase, an intestinal bacteria-produced enzyme that exerts negative impact on host fat digestion and utilization.

From a different perspective, Niewold (2007) proposed that AGP most likely work as growth permitters (as opposed to 'promoters') by inhibiting the production and excretion of catabolic mediators from intestinal inflammatory cells such as phagocytes. Concomitant or subsequent changes in the GIT bacteria are therefore most likely the consequence of an altered condition of the GIT epithelium wall. Niewold (2007) stated that this basic mechanism potentially offers an explanation for the highly reproducible effects of AGP, as opposed to those obtained by some alternatives aimed at microbiome management, hence argued that the search for alternatives could be aimed at non-antibiotic compounds with an effect on the inflammatory system similar to that of AGP.

The advent of more rapid, detailed and cheaper microbial enumeration and characterization technologies has allowed a closer examination of the effects of antibiotics to be examined in closer scrutiny. This is important because, at least in part, the addition of antibiotics to feed introduces a selective pressure that may lead to lasting changes in commensal microorganisms. Furthermore, reservoirs of antibiotic resistance genes have been shown to be stable in bacterial communities, even in the absence of antibiotics (Looft et al., 2012). Therefore, and whilst bacteria that inhabit the GIT are important for the maintenance of host health, structure and function (as described previously), contrary to these benefits, the GIT microbiota may antagonize future disease treatment by facilitating the dissemination of resistance alleles across distantly related organisms (Looft et al., 2012), and possibly into the food chain. This adds further to the pressure on the continued use of antibiotics, as discussed previously.

In an attempt to evaluate the effects of in-feed antibiotics at a more detailed level and provide insights into further mechanisms of action, Looft et al. (2012) fed ASP250 [an antibiotic feed additive containing chlortetracycline, sulfamethazine, and penicillin commonly given to pigs for the treatment of bacterial enteritis and for increased feed efficiency (chlortetracycline 100 g/ton, sulfamethazine 100 g/ton, penicillin 50 g/ton)] to young pigs for 3 weeks after weaning. Phylotyping, metagenomic, and parallel quantitative PCR (qPCR) approaches were used to track changes in microbial membership and encoded functions, enabling the detection of so-called "collateral" effects of antibiotics (i.e., effects outside of the intended growth promotion and disease prevention). These collateral effects included increases in both *Escherichia coli* populations and in the abundance of certain antibiotic resistance genes. This occurred even above a high inherent background of resistance, and many of these were likely enriched because of direct interaction with the antibiotics in ASP250. Additionally, analysis of the metagenomes implicated functions potentially involved with improved feed efficiency with the use of ASP250 (Looft et al., 2012).

In another study, Holman and Chénier (2014) evaluated the effect of the continuous administration of sub-therapeutic concentrations of tylosin and chlortetracycline on the fecal microbiota of swine through the production cycle, using 16S rRNA gene Illumina-based sequencing. The authors concluded that tylosin given at sub-therapeutic concentrations caused changes in the fecal microbiota that were identifiable at the phylum through genus levels. On the other hand, chlortetracycline had relatively minor effects in comparison, but alterations

were noticeable in specific taxa. Holman and Chénier (2014) noted that the microbiota demonstrated considerable resilience to antibiotic perturbation as most changes to the relative abundance of specific taxa were temporary. Unsurprisingly, suckling piglets had a microbial community that was very different from that of the post-weaning phase, however once established, the GIT microbiota after weaning was significantly more stable in terms of community membership. These findings confirm that dietary manipulations in the peri-weaning period are likely to have the most impact on the swine GIT microbiota throughout the production cycle.

These studies, along with numerous others, indicate that the period around weaning is particularly sensitive to perturbations in the composition and diversity of the GIT microbiota, therefore it is no surprise that detailed attention is focussed here with respect to the examination of alternative strategies for the replacement of antibiotics.

Alternative Gut Health Modulating Strategies

It is questionable whether any single feed additive or feeding strategy that could replace the roles and functions of antibiotics that, as explained above, have profound effects both directly and indirectly in the GIT to exert their effects. Table 1 describes a recent summary of the dietary options available to the pork industry to replace antibiotics (predominately AGPs) in diets and (or) water systems. There is a plethora of papers, review articles, podcasts, magazine articles, on-line publications and so on addressing these as the industry deals with the changing regulations regarding antibiotic use. It is simply outside the scope of this paper to summarize each of the mechanisms of action of these strategies, however it is pertinent to point out that some countries have been dealing without the use of AGPs in diets for more than 30 years (Sweden, 1986). Consequently, antimicrobial use in animal production in Sweden is among the lowest in Europe and has decreased by 65% over this period (Backhans et al., 2016). Improvements in biosecurity, management, feeding and disease control have assisted in keeping use low, but nevertheless, antibiotic use on Swedish pig farms does vary considerably between farms. Recently, to reduce therapeutic/prophylactic use of antibiotics even further, Backhans et al. (2016) reported that factors influencing antimicrobial use in Swedish farrow-to-finish pig farms were related to individual farmer characteristics such as age, gender and years of experience more than production-related factors. However, under Swedish circumstances, biosecurity level had no additional effect on AM use. Backhans et al. (2016) commented that this indicates the importance of the herd veterinarian's communication skills to ensure correct treatment of sick animals. Other studies in Denmark, Belgium and The Netherlands confirm the importance of management in reducing antibiotic use, therefore in some cases the use of feed additives may have little or no effect under good management conditions. Indeed, Melliere et al. (1973) reported a lesser response to antibiotics in research facilities versus commercial facilities, and that the magnitude of the response to tylosin diminished as pigs performed better (Figure 1). It would be interesting to see if similar effects occur with alternatives to AGPs in diets for pigs.

Table 1. Dietary ingredients and nutrients, dietary/feeding strategies and feed additives examined for improvement of pig health and performance in the absence of antimicrobials[1]

Functional feed ingredients and nutrients	Diet formulation and feeding strategies	Feed additives
Spray-dried plasma	Low-protein diets	Organic acids
Alternative fiber sources	Limit feeding	Inorganic acids
Conventional egg products	Fermented liquid feed	Mannan oligosaccharides
Immune egg products	Minimal diet buffering capacity	Fructo-oligosacharides
Milk protein products	Minimal antinutritional factors	Supra-nutritional levels of zinc
Lactose		Supra-nutritional levels of copper
Polyamines		Omega-3 fatty acids
Fermented soy products		Direct-fed micobials (probiotics)
Butryic acid		Prebiotics
Gluconic acid		Yeast and yeast products
Lactic acid		Bacteriocins
Glutamine		Bacteriophages
Threonine		Antimicrobial peptides
Cysteine		Conventional and recombinant enzymes
Nucleotides		Lysozyme
Medium-chain fatty acids		Egg yolk antibodies
		Essential oils
		Botanical herbs and spices
		Clay minerals
		Rare earth elements

[1]From *National Hog Farmer*, 11th April 2017 (article, "Metabolite changes in the gut rule antibiotic impact"; By Michaela Trudeau, Chi Chen, Gerald Shurson, University of Minnesota Department of Animal Science; and Fernando Leite, Richard Isaacson and Pedro Urriola, University of Minnesota Department of Biomedical Veterinary Science) (originally adapted from de Lange et al., 2010; Liu, 2015).

Moreover, any single feed additive that is intended to wholly replace the role of antibiotics in farm animals may be subject to the intense scrutiny that antibiotics have been subjected to. For example, there is evidence that heavy metals (Zn, Cu) can induce resistance in some bacteria (e.g., Yazdankah et al., 2014), leading to bans on their use in some countries and discussions in Europe currently about the future use of ZnO. Some probiotics have also been shown to harbour antibiotic resistance genes. In this regard, antibiotic resistance in some microbes does not constitute a safety concern in itself, when mutations or intrinsic resistance mechanisms are responsible for the resistance phenotype, and some probiotic strains with intrinsic antibiotic resistance could in fact be useful for restoring the GIT microbiota after antibiotic treatment (Gueimonde et al., 2013). However, specific antibiotic resistance determinants carried on mobile genetic elements, such as tetracycline resistance genes, constitute a reservoir of resistance for potential food or GIT pathogens, thus representing a potentially serious safety issue (Gueimonde et al., 2013).

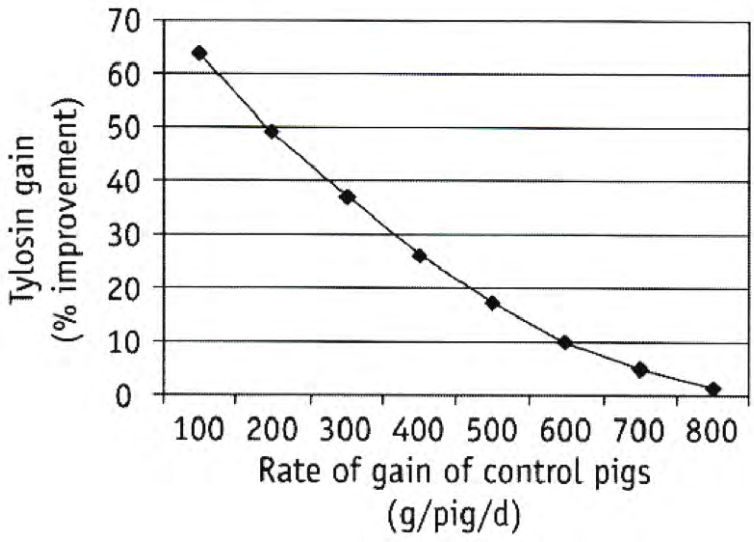


Figure 1. The impact of the performance of control animals on the magnitude of the response to tylosin in pigs (from Melliere et al., 1973).

As has been remarked already, and because the production and (or) health (through disease mitigation) benefits proven from feeding antibiotics is achieved through many different effects on the GIT, the strategy for replacing them will depend on a combination of nutritional, management, housing, health and (or) husbandry factors (Pluske, 2013). However, and as is also well recognized, there is inconsistency in the experimental and (or) commercial outcomes of many alternatives evaluated, which makes it difficult to judge their efficacy or otherwise.

In an article in *National Hog Farmer* (http://www.nationalhogfarmer.com/animal-health/evaluation-sub-therapeutic-growth-promotion-antibiotic-alternatives), Wes Schweer and Nicholas Gabler (Iowa State University, Department of Animal Science) remarked that the nature of the complexity of these alternatives creates challenges in designing and conducting experiments that will provide meaningful and repeatable results. For example, when AGP alternatives are being evaluated, it is important to know the age, health status of the pigs, the conditions under which they are raised, the composition of the diet, etc., but when this information is not provided, it is difficult to interpret results and even more difficult to compare results across studies and locations. It is also difficult to translate the results to commercial conditions, which themselves often vary widely in health status and stocking density. To address this, the National Pork Board Checkoff program has funded a project involving collaboration between Iowa State University and the USDA National Animal Disease Center for the development and validation of effective research protocols for AGP alternatives. Some of these data will be presented at this meeting.

In an extensive article examining a wide array of alternatives to antibiotics, Cheng et al. (2014) commented that ideal alternatives should:

(i) have non-toxic or no side effects on animals,
(ii) be easy to eliminate from the body or consist of short term of residues,
(iii) not induce bacterial resistance,
(iv) be stable in the feed and animal gastrointestinal tract,
(v) be easily decomposed and not affect the environment,

(vi) not affect palatability,
(vii) not destroy the normal intestinal flora of animals,
(viii) kill or inhibit the growth of pathogenic bacteria,
(ix) enhance the body resistance to the disease,
(x) improve feed efficiency and promote animal growth, and
(xi) have good compatibility.

It is apparent that there are no alternatives to antibiotics (AGPs) that currently meet all of these requirements, at least as stipulated by these authors. In the case of immunomodulators (immunostimulants), for example, a wide assortment of different products with an equally wide array of mechanisms of action exists (Table 2), that may have effects to (non-specifically) enhance the innate immune function and improve the host's resistance to diseases and (or) challenges (Cheng et al., 2014). With ZnO, there appears to be no consensus on this product's precise modes of action, which have been proposed as decreasing intestinal permeability and thereby preventing translocation of pathogenic bacteria through the intestinal barrier, to anti-inflammatory effects, through to effects on metabolic hormone status, and (or) via effects on the GIT bacteria. Despite this, it is generally acknowledged that ZnO is an important additive in nursery diets for the control of diarrhoea and promotion of growth and feed efficiency, and certainly in the absence of AGPs, any absence of ZnO (at pharmacological levels) in diets may further exacerbate the post-weaning malaise.

Table 2. A classification of immunostimulants for the pork industry (after Cheng et al., 2014).

Category	Variety
Mineral substances	Selenium, zinc, etc.
Vitamins	Vitamin A, vitamin E, vitamin C, etc.
Amino acids	Arginine, leucine, ubenimex, etc.
Chinese herbal medicines	*Astragalus*, *Echinacea*, etc.
Plant polysaccharides	*Astragalus* polysaccharide, lentinan, algal polysaccharides, ganoderan, *Polyporus* polysaccharide, chitosan, etc.
Oligosaccharides	Mannan-oligosaccharides, fructooligosaccharide, etc.
Microbial preparations	BCG vaccine, corynebacterium seedlings, *Lactobacillus*, cholera toxin B subunit, *Mycobacterium phlei*, muroetasin, prodigiosin, etc.
Immunologic adjuvants	Aluminum adjuvant, propolis, liposome, Freund's adjuvant, etc.
Hormones and hormone-like substances	Growth hormone, thymosin, metallothionein, thymopentin, etc.
Nucleic acid preparations	Polynucleotide, immune ribonucleic acid, etc.
Anthelmintics	Levomisole, metronidazole, etc.
Chemical synthetics	Levomisole, cimetidine, sodium houttuyfonate, imiquimod, pidotimod, ubenimex, tilorone, polyinosinic acid, etc.
Bacterial extracts	β-Glucan, peptidoglycan, lipopolysaccharide, etc.
Biological (cytokines)	Interferon, transfer factor, interleukin, immune globulin, etc.
Others	Bee pollen, bursa extracts, gamma globulin, heat shock protein, poly IC, glycyrrhizin, etc.

Another group of additives that has received much attention are probiotics (direct fed microbials), with once again there being a surfeit of information pertaining to their use in the peri-weaning period. Research facility and field experience, however, shows variable and inconsistent results with their use to promote growth and (or) mitigate diarrhoea in the post-weaning period. This in part is due to less than optimal experimental design in some comparisons (e.g., no Positive control treatment, an inappropriate Positive control treatment, no Negative control treatment), differences in measurements between studies that are taken and so on, however the inconsistency in reports also highlights the need for more research to better understand the mode of action and molecular mechanisms underlying probiotic effects. Probiotic bacteria are live organisms with dynamic changes in their metabolism, and phenotypes of a particular probiotic are subject to the influence by many internal and external factors that will affect the efficacy of probiotics. The identification of relationships among these factors is required for effective use and development of novel probiotics (Cheng et al., 2014).

Conclusions

De Lange et al. (2010) observed that a large amount of research has already been conducted evaluating the impact of a wide range of feed ingredients and feed additives on various aspects of GIT health and development in pigs, especially after weaning, in order to improve growth performance around this time while minimizing the use of antibiotics. A better understanding of the mechanisms whereby nutrients, feed ingredients and feed additives influence animal physiology will lead to the development of robust and field-proven alternatives to in-feed antibiotics. In this regard, de Lange et al. (2010) surmised that given the considerable advances made in the understanding of intestinal nutrient utilization and metabolism, a complimentary goal in nutrition might be to formulate young pig diets with the specific task of optimizing the growth, function and health of the GIT. Nevertheless, more research is required to optimize the pigs' response to these feed additives under varying conditions. A key concern with several of these additives is their effective delivery to the targeted location in the pig's GIT, therefore a combination of different approaches may provide the most effective alternative to infeed antibiotics.

References

Anderson, D.B., V.J. McCracken, R.I. Aminov, J.M. Simpson, R.I. Mackie, M.W.A. Verstegen and H.R. Gaskins. 1999. Gut microbiology and growth-promoting antibiotics in swine. Pig News Info. 20 115N–122N.

Backhans, A., M. Sjölund, A. Lindberg and U. Emanuelson. 2016. Antimicrobial use in Swedish farrow-to-finish pig herds is related to farmer characteristics. Porcine Health Manag. 2 18.

Cheng, G, H. Hao, S. Xe, X. Wang, M. Dai, L. Huang. and Z. Yuan. 2014. Antibiotic alternatives: the substitution of antibiotics in animal husbandry? Front. Microbiol. 5 1-15.

Coates, M.E., M.K. Davies and S.K. Kon. 1955. The effect of antibiotics on the intestine of the chick. Br. J. Nutr. 9 110–119.

Coates, M.E., R. Fuller, G.F. Harrison, M. Lev. and S.F. Suffolk. 1963. Comparison of the growth of chicks in the Gustafsson germ-free apparatus and in a conventional environment, with and without dietary supplements of penicillin. Br. J. Nutr. 17 141–151.

Collier, C.T., M.R. Smiricky-Tjardes, D.M. Albin, J.E. Wubben, V.M. Gabert, B. Deplancke, D. Bane, D.B. Anderson and H.R. Gaskins. 2003. Molecular ecological analysis of porcine ileal microbiota responses to antimicrobial growth promoters. J. Anim. Sci. 81 3035–3045.

De Lange, C.F.M., J.R. Pluske, J. Gong and M. Nyachoti. 2010. Strategic use of feed ingredients and feed additives to stimulate gut health and development in young pigs. Livest. Sci. 134 124-134.

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

Frankel, W.L., W. Zhang, A. Singh, D.M. Klurfeld, S. Don, T. Sakata, I. Modlin and J.L. Rombeau. 1994. Mediation of the trophic effects of short-chain fatty acids on the rat jejunum and colon. Gastroenterology 106 375–380.

Gaskins, H.R., C.T. Collier and D.B. Anderson. 2002. Antibiotics as growth promotants: Mode of action. Anim. Biotechnol. 13 29–42.

Gueimonde, M., B. Sánchez, C.G. de los Reyes-Gavilán and A. Margolles. 2013. Antibiotic resistance in probiotic bacteria. Front. Microbiol. 4 1-6.

Holman, D.B. and M.R. Chénier. 2014. Temporal changes and the effect of subtherapeutic concentrations of antibiotics in the gut microbiota of swine. FEMS Microbiol. Ecol. 90 599–608.

Lin, J. 2014. Antibiotic growth promoters enhance animal productivity by targeting intestinal bile salt hydrolase and its producers. Front. Microbiol. 5 1-4.

Liu, Y. 2015. Fatty acids, inflammation and intestinal health in pigs. J. Anim. Sci. Biotechnol. 6(1) 41.

Looft, T, T.A. Johnson, H.K. Allen, D.O. Bayles, D.P. Alt, R.D. Stedtfeld, W.J. Sul, T.M. Stedtfeld, B. Chai, J.R. Cole, S.A. Hashsham, J.M. Tiedje and T.N. Stanton. 2012. In-feed antibiotic effects on the swine intestinal microbiome. Proc. Natl. Acad. Sci. USA 109 1691-1696.

Melliere, A.L., H. Brown and R.R. Rathmacher. 1973. Finishing swine performance and responses to tylosin. J. Anim. Sci. 37 286. (Abstract).

Pluske J. R. 2013. Feed- and feed additives-related aspects of gut health and development in weanling pigs. J. Anim. Sci. Biotech. 4:1.

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.

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 2382–2390.

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

Yazdankah, S., K. Rudi and A. Bernhoft. 2014. Zinc and copper in animal feed – development of resistance and co-resistance to antimicrobial agents in bacteria of animal origin. Microb. Ecol. Health Dis. 25 25862.

Immuno-Epigenetics: A Veterinarian's Perspective on Reducing the Use of Antimicrobials through the Use of Nutritional Immunology

Immuno-épigénétique : la perspective d'un vétérinaire dans la réduction de l'utilisation des antimicrobiens par l'utilisation de l'immunologie nutritionnelle

Juan Carlos Rodriguez-Lecompte[1]

[1] Associate Professor of Veterinary Immunology and Diseases of Poultry, Atlantic Veterinary College, Department of Pathology and Microbiology, University of Prince Edward Island, PE, Canada, C1A 4P3, jrodriguez@upei.ca

Abstract

Reducing the use of antimicrobials in the rearing of poultry can be achieved by enhancing the natural immunity of chickens, thus minimizing disease and pathogen shedding. Intervention of the immune system of the chick in ovo has a dramatic effect on the immunity of the bird post-hatch, increasing its ability to resist a disease challenge. However, it is not only the chick's immune system that must be strengthened but also that of the adult bird. The nutritional status of the laying hen directly influences the nutrient composition of the egg and, in turn, the development and nutritional status of the embryo and hatchling. This has implications for epigenetic regulation of gene expression allowing dividing cells to memorize, or imprint, signalling events that occurred earlier in their development. Folic acid (FA), also known as folate, plays a critical role in nucleic acid and protein synthesis; a deficiency of folate significantly alters the immune response. The body utilizes FA as a methyl group donor that can be incorporated into the DNA and potentially affect gene expression. Toll like receptors (TLR) and the B cell receptor (BCR) recognize antigens, and the major histocompatibility complex (MHC) is used to present the antigen to T cells to initiate an adaptive immune response. Taken together our preliminary results it is possible to infer that FA has an immunomodulatory effect on chicken B cells, possibly affecting their ability to both recognize antigen through the TLR and BCR pathways and their ability to present antigen via the MHCII presentation pathway.

Résumé

Il est possible d'utiliser moins d'antimicrobiens dans les élevages de volaille en stimulant l'immunité naturelle des poulets de façon à réduire au maximum la morbidité et l'excrétion d'agents pathogènes. L'intervention sur le système immunitaire du poussin dans l'oeuf a un effet déterminant sur l'immunité de l'oiseau après l'éclosion en améliorant son aptitude à résister à une exposition. Cependant, il ne suffit pas de renforcer le système immunitaire du poussin, mais il faut également intervenir sur celui de l'oiseau adulte. L'état nutritionnel de la pondeuse influence directement la composition en nutriments de l'oeuf et, donc, le développement et l'état nutritionnel de l'embryon et du poussin. Cela a des répercussions sur la régulation épigénétique de l'expression des gènes permettant aux cellules de mémoriser, au moment de la division, les événements de signalisation survenus précédemment dans le développement. L'acide folique,

également appelé folates, joue un rôle essentiel dans la synthèse des acides nucléiques et des protéines; une carence en folates modifie significativement la réponse immunitaire. L'organisme utilise les folates comme donneurs de groupes méthyles pouvant être incorporés à l'ADN et pouvant potentiellement influencer l'expression génique. Les récepteurs Toll et le récepteur de lymphocytes B reconnaissent les antigènes, et le complexe majeur d'histocompatibilité (CMH) est utilisé pour présenter l'antigène aux cellules T afin d'amorcer une réponse immunitaire adaptative. Considérant l'ensemble de nos résultats préliminaires, on peut supposer que l'acide folique exerce un effet immunomodulateur sur les lymphocytes B du poulet, intervenant possiblement sur leur aptitude à reconnaître l'antigène par les voies d'exposition aux récepteurs Toll et de lymphocytes B et sur leur aptitude à présenter l'antigène par le CMH de classe II.

Introduction

Antimicrobial growth promoters (AGP) have been added to poultry feeds in low, sub therapeutic amounts for over five decades. The AGP, used as feed additive, demonstrated improvement in growth and feed efficiency in animals. The mechanism proposed to explain the AGP-mediated growth enhancement is that the intestinal microflora depresses animal growth; consequently AGP activity is based on its antibiotic properties. Nevertheless, lately the use of AGP has been examined for their potential development of antibiotic-resistant human pathogenic bacteria after long continuous use. Therefore, it is important to find potential non-pharmacological alternatives to antibiotics with similar or better properties. New alternatives include the use of enteric microflora conditioners such as probiotics, prebiotics, microflora enhancers, enzymes, acidifiers, immunomodulators and herbal products.

The current legislative climate in Canada and other countries is to use nonn-therapeutic means to prevent disease. This means that traditional drug based management strategies are no longer acceptable. With the increased trend in food safety that includes Country of Origin Legislation and similar traceability initiatives worldwide it is essential that techniques such as nutritional intervention of the immune system be developed.

During the past five decades substantial progress has been made in various areas of commercial poultry production. The high production rate of chickens has largely depended on the use of antimicrobials in the feed to control enteric and respiratory diseases, which leads to increased morbidity, and a reduction in productivity. However, if community perceptions and public health concerns no longer make it feasible to use antimicrobials to enhance production performance, maintaining a sustainable poultry production in Canada will not be easily achievable. Nevertheless, for **veterinarians** disease must still be controlled to ensure high animal health, acceptable welfare, good performance and sustainable production. Additionally, the restricted use of antimicrobials in feed results in increased pathogen shedding and heightened concerns with food safety. Thus, the immunity of the bird must be enhanced so that disease and pathogen shedding can be minimized in the absence of antimicrobials. It is now becoming apparent that intervention of the immune system of the chick *in ovo* has a dramatic effect on the immunity of the bird post-hatch, and thus the ability to resist disease challenge. However, it is not only the chick immune system that must be strengthened but also the adult bird. In the last decade, there has been a swath of publications on pre- and probiotics in poultry but there is little mechanistic information to support the claims made by manufacturers.

Epigenetics and immune cells

One strategy to enhance the immune system is targeting immune cells involved in the capture, processing and elimination of pathogens. Examples of those cells are the antigen presenting cells (APC) such as macrophages, dendritic cells and B cells. Particularly, active B cells produce specific antibodies against pathogens that have been captured and processed for either macrophages or dentritic cells. Thus, the immunity of the bird must be enhanced so that disease and pathogen shedding can be minimized in the absence of antimicrobials. It is now becoming apparent that intervention of the immune system of the chick *in ovo* has a dramatic effect on the immunity of the bird post-hatch, and thus the ability to resist disease challenge. Another strategy is modulating the gene expression of some pathogens receptors to identify and eliminate quickly unwanted pathogens; this methodology is associate with modification in the transcription of some genes without changing their gene structure, which is part of the epigenetics.

Epigenetic refers to variations in phenotype that are not the consequence of the presence or absence of genes. Rather, it refers to variations in phenotype that are the result of environmental factors including intracellular events. A classic example in poultry is the alpha-globin "switch". During the transition from the embryo to the hatchling stage, alpha-globin peptides are modified to enable increased requirements for oxygen transport post-hatch. There is no change in the structural gene, but the phenotype (ability to transport oxygen) increases.

Nutritional-intervention

The chick embryo has a long and distinguished history as a major model system in developmental biology and has also contributed to major concepts in immunology, genetics, virology, cancer, and cell biology. The chicken is an excellent model to use for the study of B-lymphocyte development due to the presence of the Bursa of Fabricius, an organ that is dedicated specifically to B cell maturation and differentiation. The B cell expression of sIg has been conserved in birds as an essential checkpoint to colonize lymphoid follicles in the bursa, whereas B cell precursors that fail to express sIg due to non-productive V(D)J recombination are eliminated. The sIg acts as the antigen binding ectodomain of the receptor while the associated Igα/Igβ heterodimer functions to couple sIg with intracellular signal transducing enzymes.

Novel methods of providing embryo specific nutrients

The development and nutritional status of the embryo and hatchling are affected by the nutrients in the egg, which in turn are influenced by the nutritional status of the hen. This has implications for epigenetic regulation of gene expression; epigenetic effects allow dividing cells to memorize, or imprint signalling events that occurred earlier in their development. For example, the dietary provision of excess methyl donors to agouti female mice before and during pregnancy led to phenotypic shifts in the coat color of their offspring. The timely introduction of methyl donors to the nucleus (affecting gene expression) may modify methylation patterns on promoters affecting transcription. However, environmental perturbations of DNA methylation do not affect the entire genome; therefore the challenge of nutritional epigenetics is to identify epigenetically labile gene regions. Several vitamins and amino acids such as folate methionine, betaine, and choline are critical in several metabolic cellular pathways involving DNA and protein biosynthesis. However methyl groups essential for DNA and histone methylation are synthesized by one carbon metabolism

using indispensable folate cofactors. Folate plays a critical role in nucleic acid and protein synthesis by supplying one-carbon units; a deficiency of folate significantly alters the immune response.

In previous experiments a correlation between graded increases in dietary folic acid levels and indices of folate status in laying hens was demonstrated, and folate transfer to the yolk was found to be saturable. Interestingly, in collaboration with others, we have been able characterize two important FA carrier-mediated transporters associated with the regulation of intestinal folate uptake and the effect of folate supplementation on their expression. These results support transporter-regulated intestinal folate uptake representing a crucial and critical way for folate delivery to peripheral tissues in chickens. Therefore, considering the direct effect on methylation and pivotal role of folate in nucleic acid synthesis, a pilot experiment was conducted. These results suggest that regardless of normal folate deterioration in unfertilized eggs (Fig. 1), the chicken embryo is likely directing folate towards organ development and maturation instead of embryonic differentiation (before day 6) (Fig. 2).

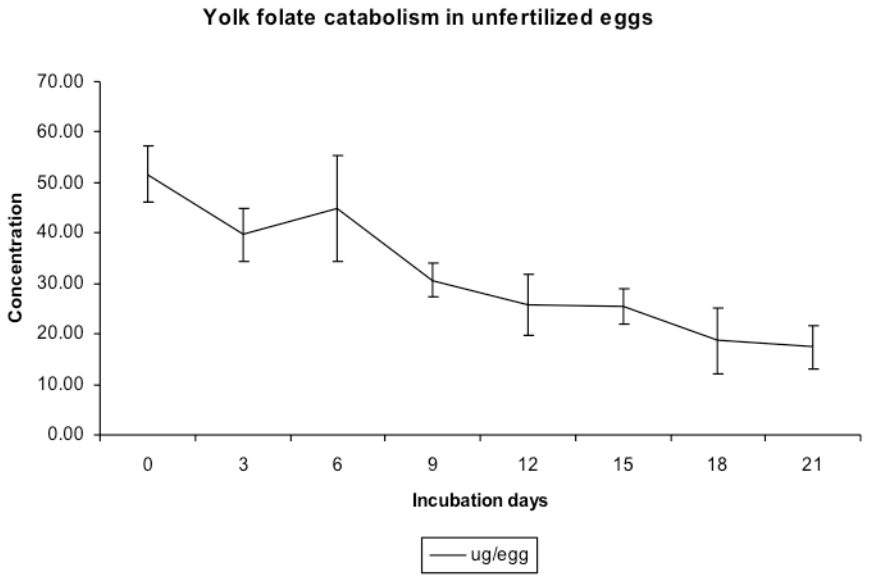


Figure 1.

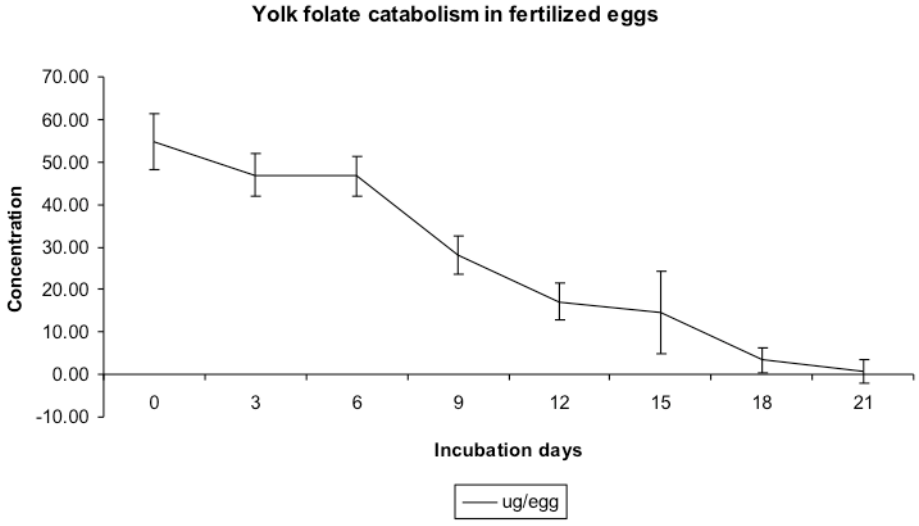


Figure 2

Epigenetics in chicken B cells

The production and migration of B cell progenitors from the bone marrow to form the bursa of Fabricius occur at embryonic day 6 (ED6). Because they are sensitive to methyl supply due to their high proliferation rate (open chromosome), characterization and evaluation of methylation patterns in BCR, and their association with the expression of anti-apoptotic genes is warranted. Specifically, there is a need to determine whether the bioactive folate is able to influence gene methylation patterns in the genes involved in the formation of BCR. Emphasis has been placed on the Igβ gene because of its essential role in intracellular BCR signalling and possible association with anti-apoptotic genes. The previous projects highlighted the need to provide dietary nutrients to modify the development of immunity. A practical way of providing these nutrients is as dietary ingredients to the hen and transference to the egg yolk during egg formation. However, there is a limit to how much can be transferred. For example, preliminary data in our laboratory demonstrated that only 10% of the folic acid fed to hens would be transferred to the egg. We have has also demonstrated that folic acid in the fertilized egg is rapidly depleted, but maturation of lymphocytes is only completed towards the end of the 21 d incubation. Clearly, supplying methyl donors to modify gene pathways for lymphocyte maturation with folic acid would be limiting because of the rapid depletion of folic acid in the yolk during embryogenesis. Thus other means of yolk nutrition are required.

In avian species, nutrient status is a central factor that contributes to the capacity of birds to respond to a pathogen challenge. Nutritional status in broiler and breeder hens influences both the innate and adaptive components of the chicken immune system. Maintenance of B-lymphocyte homeostasis requires balanced cell production, death, and proliferation. To coordinate these processes, B cells are dependent on cell extrinsic signals. Mature B cells require BCR signals and also remain sensitive to their microenvironment. In chickens the amount of post-hatching viable B cells are dependent upon the expression of BCR by embryonic bursal precursors. Although B cell apoptosis is low in the embryonic bursa, cell death increases markedly after hatching (95%). However, availability and competition for extrinsic signals regulates cellular physiology and metabolism in an analog fashion that then influences cell commitment to apoptosis or proliferation. Therefore decreases in cellular metabolism may sensitize cells to activation and action of the pro-apoptotic Bcl-2 family members, and promote apoptosis. In contrast, increases in metabolism may predispose cells to proliferate. Folate has a central role in nucleic acid and protein synthesis; therefore poor folate status significantly alters the immune response and resistance to infections in addition to influencing cell-mediated immunity in chicks. Based on these findings, we hypothesized that to maximize and protect the process of functional BCR genes, an analog control of cell physiology can be integrated with other inputs by nutrients such as folate to produce a fate decision for survival, proliferation, or apoptosis. Understanding that feeding folate alters methylation status of protein involved in the decision for B cell survival, it may increase the optimization of chicken immune system and health.

Preliminary data (Unpublished)

In vitro epigenetics in DT40 chicken B cell line

Incubation with FA was associated with changes in the methylation profile of the proximal promoter regions of TLR4, Igβ and MHCII β chain, and modified expression of TLR2b, TLR4, Igβ and MHCII β chain. However, FA concentration and exposure time seem to be integral factors in its effect. For cells not treated with lipopolysaccharide, incubation with FA caused a potential shift in either the chicken B cells ability to recognize antigen through the TLR2b pathway or the BCR. Furthermore, the inferred increase in MHCII expression may be indicative of improved antigen capture and presentation abilities by B cells. Incubation with high concentrations of FA in this experiment upregulated the MHCII β chain gene and Igβ gene while downregulating the TLR2b gene. This change in expression may fit the profile of B cells primed to present antigens to activated effector CD4+T cells. This interaction may initiate a series of activities that belong to the adaptive immune response, which includes B cell proliferation and differentiation and affinity maturation of the antibodies produced by the cell. On the other hand, this interaction will initiate cytokine production by the effector T cell, which will regulate the immune response. The changes in TLR2b, Igβ and MHCII β chain expression are not associated with changes in the proximal promoter methylation profile. FA could have affected the methylation pattern of other gene expression related sites, other epigenetic mechanisms, such as miRNA expression and histone modification, or the expression of a protein or proteins involved in regulating TLR2b, TLR4, Igβ and/or MHCII β chain.

Ex vivo epigenetics in primary DT40 chicken B cell line

Treatment with FA, while not associated with direct changes to the proximal promoter region of TLR2b, TLR4, Igβ and MHCII β chain, has affected the expression of Igβ at ED15. As Igβ is required in order to pass the selection process in the BoF, FA treatment might change the proportion of B cells that leave the BoF. It was demonstrated here that treatment with FA reduces the proportion of the population expressing medium levels of IgM, and that it is likely that the observed reduction in Igβ expression is paired with the reduction of this population, although this has to be further studied. Furthermore, FA treatment resulted in upregulation of TLR4 in embryonic B cells at day of hatch, which may have an effect on activation of the B cell in both T cell dependent and independent manners. Taken together, FA has been shown to have immunomodulatory properties affecting B cell development in the chicken embryo. Whether these changes affect the immune capabilities of the mature B cells needs to be further examined.

References

Upon request

Bovine Immunity and Strategies to Reduce Health Disorders of the Transition Cow

Immunité bovine et stratégies pour réduire les troubles de santé chez les vaches en transition

Lorraine M. Sordillo
Meadow Brook Chair Farm Animal Health and Well-Being,
College of Veterinary Medicine, Michigan State University,
East Lansing, MI, 48824, sordillo@msu.edu

Abstract

Transition cows are susceptible to increased incidence and severity of both metabolic and infectious diseases. Antibiotics are often used to treat these health problems and may contribute to the development of antimicrobial resistance on farms. Major causative factors for the increase in transition cow diseases are alterations in bovine immune mechanisms. Indeed, uncontrolled inflammation is a major contributing factor and a common link among several economically important diseases including mastitis, retained placenta, metritis, displaced abomasum, and ketosis. Dairy cow nutritional status and the metabolism of specific nutrients can regulate many immune cell functions. Thus, any disturbances in nutritional homeostasis can further enhance health disorders, increase production losses, and decrease the availability of safe and nutritious foods for a growing global population. This review will discuss the complex interactions between nutrient metabolism and immune functions in transition dairy cattle. Specifically, the ways in which altered nutrient metabolism and oxidative stress can interact to initiate and promote uncontrolled inflammatory responses in transition cows will be discussed. Understanding more about the underlying causes of dysfunctional inflammatory responses may facilitate the design of nutritional regimes that will reduce disease susceptibility in early lactation cows. Given the desire to reduce on farm use of antibiotics, nutritional-based management strategies should have a central position in any disease prevention program.

Résumé

Chez les vaches en transition, l'incidence et la gravité des maladies métaboliques et infectieuses sont exacerbées. Le recours fréquent aux antibiotiques pour traiter ces problèmes de santé peut contribuer au phénomène de l'antibiorésistance sur les fermes. Les principales causes de l'augmentation des maladies chez les vaches en transition ont pour origine des modifications dans les mécanismes de l'immunité bovine. En effet, l'inflammation incontrôlée est un facteur contributif majeur et commun pour plusieurs troubles d'importance économique, dont la mammite, la rétention placentaire, la métrite, le déplacement de la caillette et l'acétonémie. L'état

nutritionnel de la vache laitière et le métabolisme de certains nutriments peuvent réguler de nombreuses fonctions des cellules immunitaires. Par conséquent, toute perturbation de l'équilibre alimentaire peut aggraver les désordres de santé, augmenter les pertes de production et diminuer la disponibilité d'aliments sains et nutritifs pour la population mondiale en croissance. Cet revue traitera des interactions complexes entre le métabolisme des nutriments et les fonctions immunitaires chez les bovins laitiers en transition et plus spécifiquement des façons par lesquelles le métabolisme modifié et le stress oxydatif peuvent déclencher et entretenir des réponses inflammatoires incontrôlées chez les vaches en transition. Une meilleure connaissance des causes fondamentales du dérèglement des réponses inflammatoires pourrait faciliter l'élaboration de régimes alimentaires qui réduiront la sensibilité aux maladies chez les vaches en début de lactation. Dans le contexte où une diminution de l'utilisation des antibiotiques à la ferme est souhaitée, les stratégies de gestion basées sur l'alimentation devraient jouer un rôle central dans tout programme de prévention des maladies.

Introduction

Dairy cattle are susceptible to increased incidence and severity of disease during the transition period and health disorders occurring during this time are especially problematic because they greatly impact the productive efficiency of cows in the ensuing lactation (Pinedo et al., 2010). Indeed, about 75% of disease incidence within herds occurs within the first month of lactation including disorders of economic significance such as mastitis, metritis, ketosis and displaced abomasum (LeBlanc et al., 2006). As such, there have been numerous studies to better understand the underlying causes of both metabolic and infectious diseases around the time of calving in order to design more effective management practices to reduce cow health disorders during the transition period (Sordillo and Raphael, 2013). The ability of cows to resist the establishment of diseases during the transition period is related in part to the efficiency of their immune system. The immune system consists of a variety of biological components and processes that serve to protect cows from the consequences of disease. The primary roles of the immune system are to prevent microbial invasion of the body, eliminate existing infections and other sources of cellular injury, and restore tissues to normal function. The bovine immune system utilizes a multifaceted network of physical, cellular and soluble factors to facilitate defense against a diverse array of microbial challenges (Sordillo and Mavangira, 2014). This integrated system of defense mechanisms is highly regulated to maintain a delicate balance between the activation of immunity needed to prevent the establishment of disease and resolution of immune responses once the threat of invasion has passed. A poorly functioning immune system in transition cows, however, can result in a number of adverse consequences including increased disease incidence and severity that reduces the production efficiency of dairy cows. A major underlying factor that can contribute to compromised host immune defenses during the transition period is metabolic stress, which occurs when cows fail to adapt physiologically to an increase in nutrient requirements needed for parturition and the onset of copious milk synthesis and secretion (Sordillo and Mavangira, 2014). A hallmark for the onset of metabolic stress is intense lipid mobilization that can cause

excessive accumulation of plasma non-esterified fatty acids (NEFA) during negative energy balance. Metabolic stress can be characterized as resulting from the combined effects of altered nutrient metabolism associated with intense lipid mobilization, dysfunctional inflammatory responses, and oxidative stress. Together, these factors form destructive feedback loops that exacerbate metabolic stress and cause significant health disorders in transition cows (Sordillo and Mavangira, 2014). A better understanding of how nutrition and immunology interact to influence metabolic stress will facilitate the development of control programs to improve transition cow health. The ability to detect signs of metabolic stress early enough in the dry period to implement needed management adjustments prior to calving will be the key to successful monitoring and intervention programs.

Overview of the Immune System

A properly functioning immune system should protect animals from a variety of pathogenic organisms including viruses, bacteria and parasites. To accomplish this task, the immune system utilizes a complex and dynamic network of defense mechanisms that can be conveniently separate into two distinct categories: innate immunity and adaptive or acquired immunity (Table 1). Within both innate and adaptive immunity, defense mechanisms can be classified further into physical barriers, cell-mediated immunity, and soluble or humoral immunity.

Table 1. Categories of the immune system

Innate Immunity	Adaptive Immunity
Non-specific or generic response	Antigen-specific response
Immediate following exposure (minutes)	Delayed following exposure (days)
Physical & mechanical barriers	No physical/mechanical barriers
Cellular and soluble factors	Cellular and soluble factors
No immune memory	Immunological memory
Inflammation	Antibody response (vaccines)

Innate Immunity

The innate immune system is the dominant host defense mechanism in most organisms. Innate immunity includes the nonspecific components of the immune system that can respond to infectious microbes in a generic manner. Components of innate immunity constitute the first line of defense against invading pathogens since they are already present or are activated quickly at the site of exposure. Depending on the efficiency of innate defenses, microbes may be eliminated within minutes to hours following invasion. This initial line of defense can be so rapid and efficient that there may be no noticeable changes in normal physiological functions of tissues as a consequence of the attempted microbial invasion. Because of its nonspecific nature, however, innate immune mechanisms are not augmented by repeated exposure to the same insult. Components of the innate immune system include physical and mechanical barriers, phagocytes, and various soluble mediators (Table 2). Physical and mechanical barriers are essential for preventing pathogens from entering the body. Some examples of surface barrier defenses that impede microbial invasion include the skin, tears, and mucus. Once pathogens are

able to breach this initial line of defense, however, the cellular and soluble components of the innate immune response must act promptly to prevent the successful establishment of disease (Aitken et al., 2011).

Table 2. Components of innate immunity

Factor	Main Functions
Physical barriers	Block & trap microbes (skin, tears, mucus)
Pattern Recognition Receptors	Surveillance and activation of innate immune responses
Complement	Bacteriolytic & facilitates phagocytosis
Cytokines	Pro-inflammatory & immunoregulatory
Oxylipids (prostaglandins, leukotrienes)	Pro-inflammatory & pro-resolving
Endothelial Cells	Regulates leukocyte migration & activation
Neutrophils	Phagocytosis and production of ROS
Macrophages	Phagocytosis; production of cytokines and oxylipids
Dendritic Cells	Phagocytosis; links innate & adaptive immunity
Natural Killer Cells	Targets and helps to eliminate infected host cells

Adaptive Immunity

The adaptive immune response is triggered when innate immune mechanisms fail to eliminate a pathogen. The adaptive immune response is characterized by the generation of antigen-specific lymphocytes and memory cells with the ability to recognize specific antigenic determinants of a pathogen. When host cells and tissues are re-exposed to the same antigen, a heightened state of immune reactivity occurs as a consequence of immunological memory and clonal expansion of antigen-specific effector cells. A memory immune response would be much faster, considerably stronger, longer lasting, and often more effective in clearing an invading pathogen when compared to a primary immune response. In contrast to the innate immune response, adaptive immunity can take days to develop because of the clonal expansion of B and T lymphocytes specific to the invading pathogen. An amazing feature of the immune system is the ability of a host to recognize and respond to billions of unique antigens that they may encounter. It also is important that an inappropriate specific immune response does not occur against the host's own antigens. For this reason, the immune system is able to distinguish self from non-self and selectively react to only foreign antigens. Genetically diverse, membrane bound proteins called major histocompatibility complex (MHC) molecules assist in this recognition. A specific immune response will only occur if antigens are combined with an MHC molecule on the surface of certain cells, a process referred to as antigen presentation (Sordillo and Aitken, 2011). The unique features of the adaptive immune response form the basis of vaccine strategies (Table 3).

Table 3. Components of Adaptive Immunity

Factor	Main Functions
Major Histocompatibility Complex	Recognizes self from non-self
Dendritic Cells and Macrophages	Antigen presentation cells
T Lymphocytes	CD4+ Cells or T helper Cells (Th1, Th2, Th17, Treg); produce cytokines that regulate innate and adaptive immunity; immunoglobulin isotype switching
	CD8+ Cells or T cytotoxic (Tc); attacks and kills cells that express foreign antigens (virus-infected)
	□□□T Cells; prevalent at mucosal surfaces
B Lymphocytes	Antigen presentation; differentiate into antibody-producing plasma cells
Immunoglobulin (antibodies)	IgM is the largest and first produced; role in agglutination and complement activation
	IgG concentration is high in sera and is important for opsonization
	IgA is found at mucosal surfaces and has anti-viral function
	IgE is associated with allergic reactions and parasitic infections
	IgD non-secreted regulatory molecule

Periparturient Immune Dysfunction

The periparturient period is characterized as a time of dramatic changes in the efficiency of the bovine immune system. Numerous studies have documented changes in many aspects of both innate and adaptive immunity that can impact the susceptibility to new diseases in the transition cow (Aitken et al., 2011). A poorly functioning immune system can result in a number of adverse consequences. Not only are cows more likely to become infected when exposed to pathogenic organisms, but the severity of disease is also escalated. Dysfunctional inflammatory reactions that occur at both the systemic and local level, for example, are especially problematic because of the direct impact on disease pathogenesis in transition cows including metritis and mastitis. The purpose of host inflammatory responses is to eliminate the source of tissue injury, restore immune homeostasis, and return tissues to normal function. Derangements in inflammatory responses, however, can consist of a hyporesponsive state characterized by delayed migration of functionally adequate neutrophils and other innate immune factors during the early stages of disease. Conversely, the lack of an appropriate balance between the initiation and resolution of inflammation can result in an overly robust or chronic inflammatory response characterized by extensive damage to host tissues. An excellent example of the consequence of a dysfunctional immune response is the severity and duration of mastitis in early lactation cows. Studies showed that the ability of mammary glands to promptly respond to *Escherichia coli* endotoxin during early lactation when compared to cows in mid-lactation. The delayed migration of neutrophils and their reduced antimicrobial activity was suggested to be the cause of more severe coliform mastitis in the periparturient period when compared to later stages of lactation (Shuster et al., 1996).

Nutrient metabolism and dysfunctional inflammation

The underlying causes of dysfunctional inflammation during the transition period have been the subject of considerable research, with evidence to support a role for nutritional and metabolic factors. The role of negative energy balance (NEB) and changes in nutrient metabolism during the transition period has been implicated in the derangement of appropriate inflammatory responses (Sordillo and Raphael, 2013). Metabolic adaptations to NEB during early lactation result in the release of non-esterified fatty acids (NEFA) from adipose tissue stores. Cows successfully adapt to NEB when the release of NEFA is limited to concentrations that can be fully metabolized for energy needs associated with gluconeogenesis. The liver can process NEFA in several ways including oxidation for fuel, conversion back into triglycerides and stored as fat droplets in hepatocytes, or conversion to ketone compounds. Excessively high concentrations of plasma NEFA can have a detrimental effect on liver function, including triglyceride accumulation that causes fatty liver and the overproduction of ketones, such as β-hydroxybutyrate (BHB), which causes ketosis (Herdt, 2000). A detrimental cycle of dyslipidemia results in the accumulation of fat in the liver that compromises hepatocyte function, reduces gluconeogenesis, and increases the need for more lipolysis. Epidemiological studies showed that that is a positive correlation between plasma concentrations of NEFA and BHB with increased incidence of mastitis.

The impact of NEB and lipid mobilization on cells involved in the immune response was investigated. In a series of elegant studies, for example, pregnant dairy cows were mastectomized to assess the impact of milk production and NEB on various immune parameters while still maintaining the endocrine changes associated with late pregnancy and parturition (Kimura et al., 1999, Kimura et al., 2002, Nonnecke et al., 2003). The mastectomized cows experienced only moderate increases in NEFA when compared with the cows with intact mammary glands during the periparturient period. Although immune function was compromised briefly around calving in mastectomized cows, lymphocyte and neutrophil functions were diminished longer in cows with mammary glands (Kimura et al., 1999, Nonnecke et al., 2003). These authors also reported a negative impact of lactation on the composition of peripheral blood leukocyte populations (Kimura et al., 2002). The major conclusion from these studies was that the increased metabolic demands of early lactation was likely responsible for the adverse impact on immune cell populations.

Investigations into how individual metabolic components may affect immune cell populations have provided some additional insight into the linkages between metabolic and inflammatory pathways. Changes in glucose availability during the transition period may have adverse effects on some bovine immune responses. Macrophages and neutrophils require considerable energy to support antimicrobial functions and glucose serves as a primary fuel source (Calder et al., 2007). As such, dramatic decreases in blood glucose concentration during intense lipid mobilization may limit the preferred fuels needed by these immune cell population to function optimally (O'Boyle et al., 2012). The possibility exists that an activated inflammatory reaction may compete for limited nutrients with other production-related processes such as milk synthesis and

secretion. The competition for limited glucose supply may be one explanation for decreased productivity of dairy cows during disease. Evidence suggests that hyperketonemia also can adversely affect a number of important immune functions and disease susceptibility in transition cows. Ketotic cows, for example, are thought to be more susceptible to infectious diseases such as mastitis due to the detrimental impact beta-hydroxybutyrate (BHB) has on leukocyte antimicrobial mechanisms (Suriyasathaporn et al., 2000). Increased plasma BHB concentrations were also associated with reduced neutrophil antimicrobial functions during clinical cases of metritis (Hammon et al., 2006). More mechanistic studies indicated that BHB-induced oxidative stress and the subsequent activation of pro-inflammatory signaling cascades caused inflammatory injury to bovine hepatocytes (Shi et al., 2014).

Based on the current literature, progressive increases in blood NEFA concentrations before calving can be considered a significant factor impacting inflammatory responses of transition cows (Sordillo and Raphael, 2013). Both the concentration and composition of plasma NEFA change significantly in cows during periods of intense lipid mobilization. Saturated fatty acids (palmitate and stearate) and mono-unsaturated oleic acid are the predominant fats in the plasma NEFA around the time of parturition (Douglas et al., 2007, Contreras et al., 2010). Conversely, there is a decrease in some omega-3 polyunsaturated fatty acids such as eicosapentaenoic and docosahexaenoic acids. These changes are important to host inflammatory responses because the fatty acid content of immune cells will reflect compositional changes in plasma NEFA during the transition period (Contreras et al., 2010). The function of immune cells can be influenced by their fatty acid composition in several ways. For example, the relative distribution of fatty acids within the membrane phospholipids can control membrane fluidity and lipid raft formation (Raphael and Sordillo, 2013). The direct effects that intense lipolysis and changes in NEFA composition may have on specific signaling pathways in bovine immune cells have not been invested extensively. However, pro-inflammatory signaling pathways do have a clear role in bovine lipid metabolism and are associated with altered immune responses in dairy cows (Moyes et al., 2009).

Oxidative stress in transition cows

The conversion of nutrients into an energy source needed to fuel normal physiological functions occurs through a series of metabolic reactions collectively referred to as cellular respiration. Oxygen is required for aerobic cellular respiration and reactive oxygen species (ROS) are metabolites formed in the mitochondria during this as byproducts of the electron transport chain. Although the majority of ROS present in tissues result from increased cellular metabolism and energy generation, other potential sources include oxidizing enzyme pathways associated with host inflammatory responses. For example, phagocytosis is an essential part of the inflammatory response that involves the generation of toxic ROS needed to kill microbes through an oxygen-dependent mechanism. The NADPH oxidase system localized within the phagosomal membranes of neutrophils and macrophages generates ROS needed to destroy engulfed pathogens (Sordillo and Raphael, 2013).

The production of low to moderate amounts of ROS is essential for the regulation of normal cellular processes including those that regulate inflammation. Although some ROS production is optimal for normal cellular functions, the overproduction of ROS can cause cell and tissue injury and lead to a condition referred to as oxidative stress (Sordillo and Aitken, 2009). The amount of ROS is kept to nontoxic levels by a complex series of antioxidant mechanisms that have the capability to quench oxidants directly or form part of enzymatic redox couples that covert ROS to less reactive metabolites. Some examples of antioxidant that act as radical scavengers include tocopherols, ascorbic acid, carotenoids, lipoic acid and glutathione. Important ROS detoxifying enzyme systems include catalase, superoxide dismutase and the selenium-dependent antioxidant enzymes glutathione peroxidase and thioredoxin reductase (Sordillo and Aitken, 2009). During the transition period, however, the total antioxidant potential in the sera of dairy cows is diminished and ROS accumulation occurs resulting in oxidative stress (Bernabucci et al., 2002, Sordillo et al., 2007). Cellular fatty acids are the primary targets of ROS oxidation, and oxidative stress is often measured by the formation of plasma lipid hydroperoxides that are also highly reactive metabolites. Studies transition cows suggest that increased plasma NEFA concentrations can contribute to enhanced ROS production and further increase the formation of lipid hydroperoxides that exacerbate oxidative stress (Bernabucci et al., 2005). Enhanced oxidative stress may then cause additional lipolysis and that can contribute to higher plasma NEFA concentrations in transition cows (Sordillo and Raphael, 2013). Both increased plasma NEFA concentrations and lipid hydroperoxides formation are hallmarks of metabolic stress and represent important risk factors for health disorders in transition cows, such as ketosis, fatty liver, mastitis and retained placenta (Herdt, 2000, Sordillo and Raphael, 2013). Oxidative stress in dairy cattle is also thought to be a contributing factor of dysfunctional inflammatory responses especially during the transition period (Sordillo and Aitken, 2009). Oxidative stress increases inflammation by constantly activating redox-sensitive transcription factors such as NF□B, which leads to increased expression of pro-inflammatory mediators that can cause damage to host tissues. Thus, oxidative stress forms a common link between increased lipolysis during NEB and dysfunctional inflammatory responses that together form destructive feedback loops resulting in metabolic stress during the transition period (Sordillo and Mavangira, 2014).

Conclusion

Optimal host defenses against transition cow diseases occur when immune mechanisms are tightly regulated to effectively eliminate the injurious insults and return the immunes system to homeostasis. Rapid resolution of microbial infections will eliminate bystander tissue damage and prevent any noticeable changes to the productive efficiency of the cow. Whereas antibiotic therapy remains the mainstay for the treatment of many infectious diseases, there is a need for alternative and adjunct therapeutic options that target host immune responses to prevent diseases from occurring. The development of innovative strategies that can enhance an otherwise impaired bovine immune response could have a major impact on the incidence of transition cow health disorders. The challenge that confronts researchers now is to gain a better understanding of the complex

interactions between the pathogenesis of bacteria, host responses needed to eliminate the pathogens from host tissues, and methods to enhance the immune potential of before disease is established.

References

Aitken, S. L., C. M. Corl, and L. M. Sordillo. **2011**. Immunopathology of mastitis: insights into disease recognition and resolution. J Mammary Gland Biol Neoplasia 16(4):291-304.

Bernabucci, U., B. Ronchi, N. Lacetera, and A. Nardone. **2002**. Markers of oxidative status in plasma and erythrocytes of transition dairy cows during hot season. J Dairy Sci 85(9):2173-2179.

Bernabucci, U., B. Ronchi, N. Lacetera, and A. Nardone. **2005**. Influence of Body Condition Score on Relationships Between Metabolic Status and Oxidative Stress in Periparturient Dairy Cows. Journal of dairy science 88(6):2017-2026.

Calder, P. C., G. Dimitriadis, and P. Newsholme. **2007**. Glucose metabolism in lymphoid and inflammatory cells and tissues. Curr Opin Clin Nutr Metab Care 10(4):531-540.

Contreras, G. A., N. J. O'Boyle, T. H. Herdt, and L. M. Sordillo. **2010**. Lipomobilization in periparturient dairy cows influences the composition of plasma nonesterified fatty acids and leukocyte phospholipid fatty acids. J Dairy Sci 93(6):2508-2516.

Douglas, G. N., J. Rehage, A. D. Beaulieu, A. O. Bahaa, and J. K. Drackley. **2007**. Prepartum Nutrition Alters Fatty Acid Composition in Plasma, Adipose Tissue, and Liver Lipids of Periparturient Dairy Cows. J. Dairy Sci. 90(6):2941-2959.

Hammon, D. S., I. M. Evjen, T. R. Dhiman, J. P. Goff, and J. L. Walters. **2006**. Neutrophil function and energy status in Holstein cows with uterine health disorders. Vet Immunol Immunopathol 113(1-2):21-29.

Herdt, T. H. 2000. Ruminant adaptation to negative energy balance. Influences on the etiology of ketosis and fatty liver. The Veterinary clinics of North America. Food animal practice 16(2):215-230, v.

Kimura, K., J. P. Goff, and M. E. Kehrli, Jr. 1999. Effects of the presence of the mammary gland on expression of neutrophil adhesion molecules and myeloperoxidase activity in periparturient dairy cows. J Dairy Sci 82(11):2385-2392.

Kimura, K., J. P. Goff, M. E. Kehrli, Jr., J. A. Harp, and B. J. Nonnecke. **2002**. Effects of mastectomy on composition of peripheral blood mononuclear cell populations in periparturient dairy cows. Journal of dairy science 85(6):1437-1444.

LeBlanc, S. J., K. D. Lissemore, D. F. Kelton, T. F. Duffield, and K. E. Leslie. **2006**. Major advances in disease prevention in dairy cattle. Journal of dairy science 89(4):1267-1279.

Moyes, K. M., J. K. Drackley, D. E. Morin, M. Bionaz, S. L. Rodriguez-Zas, R. E. Everts, H. A. Lewin, and J. J. Loor. **2009**. Gene network and pathway analysis of bovine mammary tissue challenged with Streptococcus uberis reveals induction of cell proliferation and inhibition of PPARgamma signaling as potential mechanism for the negative relationships between immune response and lipid metabolism. BMC Genomics 10:542.

Nonnecke, B. J., K. Kimura, J. P. Goff, and M. E. Kehrli, Jr. 2003. Effects of the mammary gland on functional capacities of blood mononuclear leukocyte populations from periparturient cows. Journal of dairy science 86(7):2359-2368.

O'Boyle, N. J., G. A. Contreras, S. A. Mattmiller, and L. M. Sordillo. **2012**. Changes in glucose transporter expression in monocytes of periparturient dairy cows. J Dairy Sci 95(10):5709-5719.

Pinedo, P. J., A. De Vries, and D. W. Webb. **2010**. Dynamics of culling risk with disposal codes reported by Dairy Herd Improvement dairy herds. Journal of dairy science 93(5):2250-2261.

Raphael, W. and L. M. Sordillo. **2013**. Dietary polyunsaturated fatty acids and inflammation: the role of phospholipid biosynthesis. Int J Mol Sci 14(10):21167-21188.

Shi, X., X. Li, D. Li, Y. Li, Y. Song, Q. Deng, J. Wang, Y. Zhang, H. Ding, L. Yin, Z. Wang, and G. Liu. **2014**. beta-Hydroxybutyrate Activates the NF-kappaB Signaling Pathway to Promote the Expression of Pro-Inflammatory Factors in Calf Hepatocytes. Cell Physiol Biochem 33(4):920-932.

Shuster, D. E., E. K. Lee, and M. E. Kehrli, Jr. 1996. Bacterial growth, inflammatory cytokine production, and neutrophil recruitment during coliform mastitis in cows within ten days after calving, compared with cows at midlactation. Am J Vet Res 57(11):1569-1575.

Sordillo, L. M. and S. L. Aitken. **2009**. Impact of oxidative stress on the health and immune function of dairy cattle. Veterinary Immunology and Immunopathology 128(1-3):104-109.

Sordillo, L. M. and S. L. Aitken. 2011. Mammary Resistance Mechanisms - Endogenous. Pages 386-391 in Encyclopedia of Dairy Sciences. Vol. 3. Second ed. J. W. Fuguay, P. F. Fox, and P. L. H. McSweeney, ed. Academic Press, San Diego.

Sordillo, L. M. and V. Mavangira. **2014**. The nexus between nutrient metabolism, oxidative stress and inflammation in transition cows. Anim Prod Sci 54(9):1204-1214.

Sordillo, L. M., N. O'Boyle, J. C. Gandy, C. M. Corl, and E. Hamilton. **2007**. Shifts in thioredoxin reductase activity and oxidant status in mononuclear cells obtained from transition dairy cattle. J Dairy Sci 90(3):1186-1192.

Sordillo, L. M. and W. Raphael. **2013**. Significance of metabolic stress, lipid mobilization, and inflammation on transition cow disorders. The Veterinary clinics of North America. Food animal practice 29(2):267-278.

Suriyasathaporn, W., C. Heuer, E. N. Noordhuizen-Stassen, and Y. H. Schukken. **2000**. Hyperketonemia and the impairment of udder defense: a review. Vet Res 31(4):397-412.

Dairy Cow Ketosis: Novel Biomarkers for Early Detection of Disease Risk

Cétose de la vache laitière : nouveaux biomarqueurs servant à la détection précoce de risques de maladie

Burim N. Ametaj[1] and Guanshi Zhang[1,2]

[1]Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada, [2]Institute of Metabolomic Medicine, School of Medicine, University of California San Diego, La Jolla, CA, USA

Abstract

The incidence rate of periparturient diseases of dairy cows in Canada and elsewhere has increased steadily during the last two decades. In fact, the number of culled cows per dairy herd, per year in Canada has reached more than 50% with metritis, mastitis, and laminitis leading the list. Ketosis also is a major disease of dairy cows given its high incidence rate. Ketosis has been defined as a metabolic disturbance of energy metabolism that affects subclinically more than 40% of periparturient dairy cows in a herd. Another 12-15% of the cows are affected by the clinical form of the disease. This becomes economically important when taking into consideration the impact that both subclinical and clinical forms of ketosis have on the incidence rate of other periparturient diseases and in lowering milk yield. Presently, diagnosis of ketosis is performed by measurement of ketone bodies in various body fluids with blood β-hydroxy-butyric acid (BHBA) as the golden standard. Cows diagnosed with ketosis are treated with solutions containing dextrose or glucose and glucocorticoids aiming to normalize blood glucose and lower ketone bodies. Moreover, antibiotics like monensin have been approved to be used in Canada as feed additives to prevent occurrence of ketosis; however, this has raised concerns of bacterial resistance. Given the side effects of antibiotics, other more natural preventative treatments would be desirable to be developed and used in the future. However, since not all transition dairy cows are affected by ketosis it would be beneficial to diagnose cows at greater risk of developing ketosis at an earlier stage and treat only those cows with preventative interventions. This necessitates identification and application of new tests that can diagnose cows at risk of developing ketosis at the earliest possible stage of disease. Recently our lab has applied metabolomics technologies to identify new metabolite signatures that are able to indicate with very high sensitivity and specificity dairy cows at risk of developing ketosis at the beginning of dry off period. These new biomarker sets of ketosis can be used in the future to identify and treat cows at risk of developing ketosis at the earliest possible stage, prior to elevation of ketone bodies in body fluids. In this article we also will discuss a paradigm switch that is happening with regards to the methodology and the philosophy that the animal science and veterinary scientists are using in approaching the studying of causality and pathomeachisms of disease around parturition.

Résumé

L'acétonémie est le principal désordre métabolique qui touche les vaches laitières au Canada, 40 % d'entre elles souffrant de la forme subclinique et 12 % à 15 % présentant des signes cliniques de la maladie. L'acétonémie entraîne d'importantes répercussions économiques compte tenu de son effet négatif sur le taux d'incidence d'autres maladies périnatales et sur le rendement en lait. Selon la définition stricte habituellement proposée, l'acétonémie fait référence à une production excessive de corps cétoniques visant à corriger le bilan énergétique négatif créé chez la vache immédiatement après le vêlage. Présentement, le traitement de l'acétonémie vise le rétablissement des concentrations normales de glucose sanguin et la diminution de la quantité de corps cétoniques. En matière de prévention, des antibiotiques comme le monensin ont été approuvés en compléments alimentaires au Canada. Cette décision a soulevé des craintes par rapport à l'antibiorésistance. Il convient de noter que les vaches souffrant d'acétonémie sont surtout traitées pour diminuer les symptômes et non pour éliminer les causes de la maladie. Une nouvelle approche holistique apparue depuis peu est très prometteuse si on considère la façon avec laquelle elle aborde les maladies de la vache laitière périparturiente. Cette approche pluridisciplinaire appelée biologie des systèmes inclut des sciences telles que la génomique, la transcriptomique, la protéomique et la métabolomique. Cet article portera principalement sur la contribution de la métabolomique dans l'étude des causes et pathomécanismes de l'acétonémie ainsi que sur l'identification de nouveaux biomarqueurs permettant de détecter les risques de développement de la maladie avant le diagnostic de concentration élevée de corps cétoniques. Identifier les sujets à risque sera utile pour la mise au point de nouvelles technologies de prévention de l'acétonémie.

Definition of Ketosis

Traditionally ketosis has been defined as a metabolic disorder of dairy cows that occurs mostly during the first few weeks after parturition. Ketosis refers to increased concentrations of ketone bodies including acetoacetate, acetone, and beta-hydroxy-butyric acid (BHBA) in the blood, urine, or milk. It usually affects 40% or more of the cows in a herd but in some problematic herds might reach up to 80% of the cows (Gordon et al., 2013). Ketosis usually is a ‘silent’ disease because most cows do not show clinical signs.

Presently, ketosis is classified into two categories as subclinical or clinical. Classification is based mainly on the concentration of BHBA in the blood. The subclinical form of the disease is characterized by concentration of BHBA in the blood equal to or more than 1.2 mmol/L (12.4 mg/dL). On the other hand, cows that have greater than 3.0 mmol/L (30.9 mg/dL) of BHBA in the blood are considered clinically affected by ketosis (Oetzel, 2004). The incidence rate of clinical ketosis ranges between 2-15% during the first 6 wks after parturition (Gordon et al., 2013). The most typical signs of disease are anorexia, decreased dry matter intake, weight loss, selective intake of hay, weak ruminal contractions, dry feces, and depression. Moreover, some of the cows might experience signs of nervous dysfunction, abnormal licking, and uncoordinated gate or aggression. It should be noted that in some cases although cows have very high ketone bodies they do not show clinical signs of disease. Some other cows with lower level of ketones might show clinical signs of ketosis. Generally, ketones are used as an energy source from various organs including brain.

Pathobiology of Ketosis

The traditional (i.e., reductionist) approach to the pathobiology of ketosis has proposed that ketosis is a consequence of establishment of a negative energy balance (NEB) immediately after parturition because large amounts of energy are eliminated through milk production while the energy intake is such that it does not support the large requirements of energy (i.e., glucose) for milk production. The lack of energy triggers mobilization of non-esterified fatty acids (NEFA) from adipose tissue that are transported to the liver and partially converted into energy and ketone bodies. Liver production of ketones is considered as the endogenous pathway of ketone production. There is also a second exogenous source of ketone bodies related to rumen digestion of fibrous materials or carbohydrates. This includes acetate that serves as a precursor of ketone production in the liver and butyrate. Butyrate released in the rumen is absorbed by epithelial cells and is converted into BHBA and later released into systemic circulation. Therefore, diets high in grain are usually associated with high production of acetate and butyrate in the rumen and as a consequence contribute to the blood pool of ketone bodies.

Implications of Ketosis

Ketosis is associated with decreased milk production, poor reproductive performance, and even culling of the affected cows usually during the first month after parturition. The disease becomes important economically because it requires treatment of cows with glucose products or hormonal therapy to induce gluconeogenesis. Ketosis also has been shown to be associated with greater risk for other periparturient diseases. For instance, Suthar et al. (2013) reported that cows with subclinical ketosis have 1.5, 9.5, and 5.0 times greater odds of developing metritis, clinical ketosis, and displaced abomasum, respectively.

Ketosis Diagnostic Tests

Diagnosis of ketosis is based on measurement of ketone bodies in the blood, urine, and milk during 1-6 weeks after parturition. There are several different kits that the dairy industry is using to diagnose cows with ketosis with urine test strips dominating the market. Diagnosis of ketosis gives the dairy producer the possibility to treat dairy cows; however, it should be noted that by the time that ketosis is diagnosed, through measurement of ketones in the urine during the first 6 wks after parturition, there is no possibility to reverse the already advanced state of disease. This is associated with high veterinary bills and medication expenses costing dairy industry millions of dollars on a yearly basis.

Use of Antimicrobials for Prevention of Ketosis

Several research investigations conducted in Canada and elsewhere, during the late 80s and 90s, indicate that feeding monensin (an antibiotic) to dairy cows before parturition has lowered the incidence of subclinical ketosis, by decreasing concentration of BHBA in the blood by around 40%, during the first 3 wks of lactation (Sauer et al., 1989; Duffield and Bagg, 2000). Based on those research activities in December 1997, a controlled release capsule containing monensin was approved for use in dairy cattle in Canada as an aid to prevent subclinical ketosis. Monensin also may have several other advantages for dairy cattle, including improved energy metabolism, increased milk production, and altered milk components. However, monensin and other supplemental antibiotics known as ionophores have been associated with development of bacterial resistance. Although ionophores produced by Streptomyces species are not used for treatment of human infectious diseases feeding of antibiotics in dairy cows has already

established resistant rumen bacteria that might affect treatment of cows with other antibiotics (Russell and Houlihan, 2003). There is also a concern that the resistant genes could be transferred to other bacteria populating the gastrointestinal tract or other mucosal tissues of both human and animals' microbiomes. Indeed, there is mounting evidence that not only antibiotic resistance genes (ARGs) encountered in bacterial pathogens are of relevance, but rather, all pathogenic, commensal as well as environmental bacteria - and also mobile genetic elements and bacteriophages - form a reservoir of ARGs (the resistome) from which pathogenic bacteria can acquire resistance via horizontal gene transfer (reviewed by von Wintersdorff et al., 2016). Horizontal gene transfer has caused antibiotic resistance to spread from commensal and environmental species to pathogenic bacteria. Given the concern of researchers and customers regarding the widespread dissemination of ARGs there is an urgent need to develop new strategies for prevention of ketosis and other periparturient diseases of transition dairy cows. In this aspect identification of biomarkers for monitoring the risk of ketosis and development of alternative preventive strategies is warranted. However, before dealing with the discovery of biomarkers it is important to discuss whether ketosis is indeed a metabolic disease characterized by elevation of BHBA or other ketone bodies in the host body fluids or something more than that?

Is Ketosis a Disturbance of Only Ketone Bodies?

Ketosis has been traditionally defined as a metabolic disturbance of glucose and fat metabolism by the reductionist way of thinking. However, recent data generated by the systems biology approach have raised an important question: is ketosis a metabolic disease defined by alteration of ketone bodies alone? In fact, mounting evidence suggest that increased ketone bodies after parturition are just one small aspect of what has been defined as ketosis. Intriguingly, new evidence demonstrates that the disease is preceded and followed by many other metabolite alterations and very different metabolite signatures that cast doubts on the traditional definition of ketosis (Zhang, et al., 2017). Moreover, preketotic cows have been found to suffer from a chronic metabolic acidosis and chronic inflammatory state long before they are diagnosed with high ketone bodies (Zhang et al., 2016). Before we describe in detail these new findings let's define what is the reductionist approach to disease and what is the new philosophy of systems biology that is challenging the reductionist thinking and approach to disease that has emerged during the last decade?

The science of biology including animal and veterinary sciences have been dominated by the philosophy of reductionism starting from their establishment. This philosophy considers that the animal body is a sum of all its parts (i.e., atoms, molecules, cells, tissues, organs, and systems) and by knowing how each of those parts work separately we can deduct how the whole body functions. This philosophy has been studying the animal body by splitting it into smaller parts and has put tremendous efforts to understand how those parts work. However, although the reductionist approach has provided a detailed knowledge about the animal body as a whole it seems like this type of approach has failed to explain causality of disease and disease pathomechanisms as well as development of preventive strategies. Moreover, the reductionist approach has shown that it cannot explain the complex interactions among groups of metabolites, tissues, organs or systems as a whole and the influence of the environment on health and disease states.

A notable example to illustrate the failure of reductionist approach is the concept of 'one metabolite one disease'. For instance, the reductionist thinking has proposed that ketosis is a metabolic disorder related to establishment of NEB immediately after parturition and mobilization of NEFA from adipose stores and production of ketone bodies from the liver to provide an alternative form of energy. However, various studies have shown that providing cows more energy prior to parturition or even after parturition has not been able to prevent development of ketosis (Dann HM et al., 2005). The science of reductionism also cannot explain why only part of the cows after parturition are affected by ketosis (~40%) and not all of them, although all cows produce similar large amounts of milk during the first 6 wks after calving and all of them go through NEB. Several studies related to oral feeding of monensin (an antibiotic) has demonstrated a significant reduction in the incidence rate of subclinical ketosis up to 35%, although monensin has been shown in some studies to lower feed intake, therefore, exacerbate NEB (Sauer et al., 1989). All the aforementioned examples suggest that ketosis is not a disease related only to NEB. It should be noted that although animal health scientists have studied ketosis for more than 50 years the causality and pathobiology of the disease still remain unclear and there is no well-defined strategy for prevention of occurrence of ketosis with dietary interventions.

Based on the aforementioned challenges, a new and most recent scientific approach to ketosis suggests that the disease is far more complex than previously thought and that disease involves multiple systems including the genotype and phenotype of the cow, multiple tissues, organs and systems as well as environmental factors and multilevel interactions among all factors involved. This new methodology also indicates that the disease state is far more complex condition than simply 'one disturbed metabolite' or the simple sum of each component involved. This new philosophy of science is known as systems biology or veterinary approach that looks at disease state as a more complex condition that involves alteration of multiple metabolites, proteins, enzymes, tissues, organs, and systems and their interactions. Identification of those alterations, during the early stages of the disease process, can be used to early diagnose the disease state and even to predict the risk of developing the disease at a very early phase. Systems biology includes sciences such as genomics, transcriptomics, proteomics, and metabolomics. The latter involves identification and measurement of a whole variety of metabolite alterations or signatures that can characterize the entire disease process from early stages of disease to its resolution. These metabolite signatures are also known as biomarkers of disease.

New Biomarkers of Ketosis

Before describing different types of biomarkers, it would be of interest to define what biomarkers are. There have been various definitions of biomarkers; however, the one that is more suitable for a certain disease state is that: 'A biomarker is a characteristic that can be objectively measured and evaluated as an indicator of a physiological or a pathological process' (Jain, 2010). Known classical biomarkers of metabolic diseases in dairy cows are blood ketone bodies (BHBA) during ketosis, blood NEFA during fatty liver, and blood calcium during milk fever.

There are several different types of biomarkers of disease. We will focus on description of only three of them in this article. The first group of biomarkers are disease diagnostic biomarkers; those that give insight into the pathomechanism and pathobiology of the disease. For instance, not very much is known about the pathogenesis of ketosis. Therefore, it would be interesting to use various system biology sciences to identify as many possible biomarkers that characterize the

disease and that pinpoint the systems, organs, tissues, cells, genes, proteins, enzymes, and metabolites that are involved in the disease process. These types of biomarkers characterize the disease and might help to identify the causal agent(s) of the disease. The second type of biomarkers are predictive biomarkers. This type of biomarkers can be used to predict the risk of disease during the early stages when the disease is not symptomatic yet. This requires longitudinal and retrospective studies with collection of samples at stages when cows look clinically normal. This type of biomarkers can be used to screen or monitor cows before the disease appears clinically. They can help to make early decisions to prevent the occurrence of disease. A third type of biomarkers are prognostic biomarkers. This type of biomarkers serves to predict the outcome of the disease process or success or failure of the treatment. In the livestock industry this type of biomarkers might help producers to make important economic decisions whether it is better to cull the cow immediately or enter into a vicious circle of treatments that would end up culling the cow anyway but with extra and unnecessary expenses. So, what are some of the most recent developments in the field of metabolomics biomarkers for the risk of ketosis?

A Metabolomics Approach to Ketosis

Recent research conducted by our lab indicates that ketosis is not simply a metabolic disorder but also involves aspects of a chronic inflammatory response and chronic metabolic acidosis. In a recent article we reported that preketotic cows had presence of an activated innate immunity starting from -8 wks prior to parturition (Zhang et al., 2016). More interestingly data showed that activation of innate immunity continued also at -4 wks prior to the expected day of parturition and also during the disease diagnosis week. Additionally, preketotic and ketotic cows were in a state of metabolic acidosis with concentrations of lactate in the serum been 2.36.-, 2.07-, and 1.51-fold greater than in the healthy controls. The source of lactate is not clear at present; however, lactate has been shown to function as a negative regulator of inflammasome activator. A recent study reported that the exogenous administration of lactate inhibited the Toll-like receptor 4-dependent pro-inflammatory responses in murine and human macrophages stimulated by LPS (Hoque et al., 2014). This suggests that greater serum lactate in preketotic cows might be a host response to ease the chronic inflammatory response.

Moreover, in another article (Zhang et al., 2017) we showed that preketotic, ketotic, and even postketotic cows had alterations in multiple metabolites involved in fatty acid metabolism (acylcarnitines), phospholipids (phosphatidylcholines), sphingolipids (sphingomyelines), biogenic amines, and amino acids. In that study, we identified and quantified 128 metabolites in the serum of cows with ketosis and healthy controls (CON) using a direct injection/liquid chromatography and tandem mass spectrometry (DI/LC-MS/MS) platform (Zhang et al., 2017). Both univariate and multivariate analyses revealed significant alterations in the serum metabolic signatures in preketotic (i.e., -8 wks and -4 wks prepartum), ketotic (i.e., disease wk) and postketotic cows (+4 and +8 wks postpartum). One of the most significant findings of that study was identification of a seven-metabolite signature set for early diagnosis of ketosis at -8 wks prepartum (i.e., 9-11 wks prior to traditional diagnosis of ketosis through measurement of serum BHBA). Those 7 serum biomarkers include lysine (Lys), lysophosphatidylcholine acyl (LPC)-C17:0, -C18:0, -C16:0, isoleucine (Ile), kynurenine (Kyn), and leucine (Leu). The selection of the 7-metabolite set was based on their values of the variable importance in projection (VIP > 1.0). The biomarker model was evaluated by a receiver operating characteristic (ROC) curve,

and the area under the curve (AUC) was 1 (95% CI 1-1), which indicates that the serum biomarkers identified have excellent sensitivity and specificity and can be used to predict with high accuracy the risk of susceptibility to ketosis.

Besides the 7-serum biomarker signature reported in the paper by Zhang et al. (2017), 14 other metabolites also were altered in preketotic cows at -8 wks prepartum when compared with CON cows. Although those metabolites have relatively lower VIP values compared to the 7 highest biomarker set indicated, all of them have high individual AUC values (> 0.8), which suggest that they can serve as excellent or good predictive biomarkers for the risk of ketosis. In order to give a better view of perturbed serum metabolome in preketotic cows, the present article will re-elaborate our most significant findings based on different metabolite groups including acylcarnitines (ACs) (Figure 1), amino acids (AAs) (Figure 2), biogenic amines (BAs) (Figure 3), lysophosphatidylcholines (LPCs) (Figure 4), phosphatidylcholine diacyl (PC aa) (Figure 5), phosphatidylcholine acyl-alkyl (PC ae) (Figure 6), and sphingomyelins (SMs) (Figure 7).

Among the 20 AC standards embedded in the commercial Absolute*IDQ*® p180 Kit (BIOCRATES Life Science AG, Innsbruck, Austria), seven ACs including acetyl-L-carnitine (C2), propionyl-L-carnitine (C3), butyryl-L-carnitine (C4), valeryl-L-carnitine (C5), decanoyl-l-carnitine (C10), hexadecanoyl-L-carnitine (C16), octadecenoyl-L-carnitine (C18) were quantified in both groups of cows (Figure 1). Interestingly, preketotic cows had 3.5-fold greater levels of C10, a medium chain AC, at -8 wks before parturition than CON cows (Zhang et al., 2017). Acylcarnitines play important roles as transporters of fatty acids into mitochondria for β-oxidation (Jones et al., 2010). In human studies, elevated levels of both short- and long-chain ACs in the plasma/serum and urine have been observed during diabetic ketosis (Frohlich, et al., 1978; Genuth and Hoppel, 1979; Hoppel and Genuth, 1980). Identification of elevated serum C10 at -8 wks prepartum suggests that preketotic cows experienced a state of dysregulated fatty acid oxidation, which might be associated with the activation of pro-inflammatory signaling pathways (Rutkowsky et al., 2014). This physiological dysregulation starts far earlier (2 months prior to calving) than traditional perturbation of NEFA and ketone bodies observed after parturition.

Preketotic cows also was found to have significant alterations in the AA metabolism during the dry-off period. Eight predictive serum AAs or their catabolites including arginine (Arg), citrulline (Cit), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), and tryptophan (Trp) were identified as ketosis biomarkers at -8 wks prepartum (Figure 2). Specifically, Lys (6.16-fold), Ile (2.43-fold), and Leu (2.53-fold) were among the most dominant species of AAs that increased in the serum of preketotic cows at -8 wks before parturition and were included in the 7-metablite biomarker model (Zhang et al., 2017). Moreover, it should be noted that the three AAs (i.e., Lys, Ile, and Leu) were consistently increased in preketotic, ketotic, and postketotic cows at 5 studied time points, which indicates that these three AA biomarkers can be used to detect ketosis at subclinical stages. Beside the ketogenic AAs (e.g., Lys) and two branched chain amino acids (BCAAs, Leu and Ile), increased levels of Arg, Cit, Gly, and His, and decreased levels of Trp in the serum can be used as reliable biomarkers for prediction of risk of ketosis at -8 wks before calving. The pathobiology of altered AA metabolism in preketotic, ketotic, and postketotic cows has been discussed in detail in our recent published article (Zhang et al., 2017). It is speculated that the increased concentrations of most AAs in the serum of

preketotic cows are attributed to their roles in various anabolic/catabolic functions and most importantly in the mounting of an immune response. Indeed, preketotic and ketotic cows were in a state of 'low-grade' or 'chronic' inflammation during the dry off period as indicated by increased serum amyloid A and tumor necrosis factor in their serum (Zhang et al., 2016).

Four BAs including Kyn, acetylornithine, carnosine, and sarcosine were identified as additional predictive biomarkers for risk of ketosis in the serum of cows at -8 wks prepartum (Figure 3). In particular, Kyn was selected as one of the seven biomarker signature set based on its high VIP score (VIP > 2) (Zhang et al., 2017). One of the most significant findings with regards to BAs was that Kyn and acetylornithine are considered not only predictive biomarkers for risk of ketosis at -8 wks, but also as important metabolites for evaluating the onset and progression of ketosis from -8 wks prepartum to +8 wks postpartum. Intriguingly, Kyn, a Trp catabolite, plays a very significant role in suppressing immunity and keeping the inflammatory response under control (Mellor et al., 2013). Indeed, preketotic cows had lower concentration of Trp and greater cocnentrations of Kyn in the serum at -8 wks prepartum compared with CON cows (Zhang et al., 2017). Another important serum BA biomarker for ketosis, acetylornithine, is more related to its role in the ornithine metabolism and its indirect impact on production of polyamines like putrescine, spermidine, and spermine (Zhang et al., 2017). Polyamines have been shown to have modulatory roles on T-cell activity and inhibit synthesis and release of pro-inflammatory cytokines (i.e., TNF and IL-1) by monocytes after LPS stimulation (reviewed by Ramani et al., 2014). As such, polyamines play important roles in the control of inflammatory response.

Serum predictive biomarkers for risk of ketosis were also identified among the metabolite groups of glycerophospholipids and sphingolipids. Given the large number of metabolites quantified of the phospholipid metabolism: 77 glycerophospholipids and 14 sphingolipids (Zhang et al., 2017), we summarized phospholipid metabolites into four groups: 10 LPCs (Figure 4), 34 PCs aa (Figure 5), 33 PCs ae (Figure 6), and 14 SMs (Figure 7). Overall, 7 phospholipid species were identified to be associated with ketosis and as potential biomarkers for predicting the risk of ketsis in the serum of dairy cows. Most significantly, 3 LPC metabolites including LPC C17:0, C18:0, and C16:0 were increased 3.11-fold, 2.85-fold, and 2.5-fold, respectively, in the serum of preketotic cows at -8 wks preaprtum (Zhang et al., 2017). It should be noted that all 3 LPCs were ranked as top metabolites (VIP > 2) in the VIP plot and are included in the biomarker set that we developed for prediction of ketosis. Of note, concentration of one single LPC biomarker (i.e., lysoPC a C28:0) decreased in preketotic cows at -8 wks prepartum (Figure 4). Neither concentrations of PC aa or PC ae species in the serum showed any significant differences between the two groups of cows at -8 wks prior to parturition. The only species of PC (PC ae C44:4) increased 1.25-fold in preketotic cows and was identified as a biomarker for the risk of ketosis (Figure 5 and Figure 6). In addition, two serum biomarkers for the risk of ketosis were identified from SM species [i.e., SM (OH) C24:1 and C20:2] at -8 wks prepartum (Figure 7). Alterations of metabolites in the phospholipid metabolism and their potential association with the pathobiology of ketosis are thoroughly discussed in the article by Zhang et al. (2017). Briefly, LPC species are known as strong pro-inflammatory mediators but at certain concentrations they also can attenuate the chronic inflammatory state present in preketotic cows.

Overall, these new findings suggest that: 1) ketosis is not simply a metabolic disorder of NEFA and ketone bodies. Contrary to the traditional definition that this is simply a disorder related to

NEB and mobilization of NEFA from adipose tissue and production of ketone bodies from the liver it is obvious that the disease is preceded and associated with an inflammatory trigger and a chronic inflammatory state, 2) ketosis also is preceded and associated with a state of chronic metabolic acidosis. This is indicated by greater concentrations of lactate in the blood of preketotic (-8 and -4 wks prior to calving) and ketotic cows (the week of diagnosis of disease). It is possible that lactate might have modulatory roles on the immune response of transition dairy cows; 3) ketosis is associated with multiple alterations in serum acylcarnitines, phosopholipids, and sphingolipids. Elevated acylcarnitines are indicators of dysfunction of mitochondria and the inability to metabolize fatty acids to fully metabolized end products. Moreover, alterations in PCs suggest that preketotic and ketotic cows have perturbations of choline metabolism associated with fatty liver; 4) ketosis also is associated with major alterations in AA metabolism. The amino acids affected are involved not only in gluconeogenesis but also in supporting an immune response in those cows, 4) during ketosis multiple BAs are elevated in the blood circulation during the preketotic and ketotic periods. Some of the BA metabolites like Kyn and its pathway of metabolites as well as acetylornithine are associated with maintaining host immunity under control. Overall, these new findings further contribute to the better understanding of the etiology and pathobiology of ketosis process around parturition and could be used as predictive biomarkers of risk of ketosis. More efforts are warranted to develop new preventive strategies for prevention of disease development.

References

Alphen, P.H. Savelkoul, and P.F. Wolffs. 2016. Dissemination of Antimicrobial Resistance in Microbial Ecosystems through Horizontal Gene Transfer. Front. Microbiol. 7 173-183.

Baban. 2003. Tryptophan catabolism and T cell responses. Adv. Exp. Med. Biol. 527 27-35.

Duffield, T.F., and R.N. Bagg. 2000. Use of ionophores in lactating dairy cattle: a review. Can Vet J. 41 388-394.

Errea, A., D. Cayet, P. Marchetti, C. Tang, J. Kluza, S. Offermanns, J.C. Sirard, M. Rumbo. 2016. Lactate Inhibits the Pro-Inflammatory Response and Metabolic Reprogramming in Murine Macrophages in a GPR81-Independent Manner. PLoS One. 2016 Nov 15;11(11):e0163694.

Frohlich, J., D.W. Seccombe, P. Hahn, P. Dodek, and I. Hynie. 1978. Effect of fasting on free and esterified carnitine levels in human serum and urine: correlation with serum levels of free fatty acids and B-hydroxybutyrate. Metabolism. 27 555-561.

Genuth, S.M., and C.L. Hoppel. 1979. Plasma and urine carnitine in diabetic ketosis. Diabetes. 28:1083-1087.

Gordon, J.L., S.J. Leblanc, and T.F. Duffield. 2013. Ketosis treatment in lactating dairy cattle. Vet. Clin. North Am. Food Anim. Pract. 29 433-445.

Hoppel, C.L., and S.M. Genuth. 1980. Carnitine metabolism in normal-weight and obese human subjects during fasting. Am. J. Physiol. 238 E409-E415.

Hoque, R., A. Farooq, A. Ghani, F. Gorelick, and W.Z. Mehal. 2014. Lactate reduces liver and pancreatic injury in Tolllike receptor- and inflammasome-mediated inflammation via GPR81-mediated suppression of innate immunity. Gastroenterology 146 1763-1774.

Jain, K.K. 2010. In Jain K.K. (ed). Introduction, In The Handbook of Biomarkers. Springer, New York, 2010 pp

Oetzel, G.R. 2004. Monitoring and testing dairy herds for metabolic disease. Vet. Clin. North Am. Food Anim. Pract. 20 651-674.

Mellor, A.L., D. Munn, P. Chandler, D. Keskin, T. Johnson, B. Marshall, K. Jhaver, and B.

Russell, J.B., **and A.J. Houlihan. 2003.** Ionophore resistance of ruminal bacteria and its potential impact on human health. FEMS Microbiol. Rev. 27 65-74.

Ramani, D, J.P. De Bandt, and L. Cynober. 2014. Aliphatic polyamines in physiology and diseases. Clin. Nutr. 33 14-22.

Rutkowsky, J.M., T.A. Knotts, K.D. Ono-Moore, C.S. McCoin, S. Huang, D. Schneider, S. Singh, S.H. Adams, and D.H. Hwang. 2014. Acylcarnitines activate proinflammatory signaling pathways. Am. J. Physiol. Endocrinol. Metab. 306 E1378-E1387.

Sauer, F.D., J.K.G. Kramer, and W.J. Cantwell. 1989. Antiketogenic effects of monensin in early lactation. J. Dairy Sci. 72 436-442.

Suthar, V.S., J. Canelas-Raposo, A. Deniz, and W. Heuwieser. 2013. Prevalence of subclinical ketosis and relationships with postpartum diseases in European dairy cows. J. Dairy Sci. 96:2925-2938.

von Wintersdorff, C.J., J. Penders, J.M. van Niekerk, N.D. Mills, S. Majumder, L.B. van

Jones, L.L., D.A. McDonald, and P.R. Borum. 2010. Acylcarnitines: role in brain. Prog. Lipid Res. 49 61-75.

Zhang, G., D. Hailemariam, E. Dervishi, S.A. Goldansaz, Q. Deng, S.M. Dunn, and B.N. Ametaj. 2016. Dairy cows affected by ketosis show alterations in innate immunity and lipid and carbohydrate metabolism during the dry off period and postpartum. Res. Vet. Sci. 107 246-256.

Zhang, G., E. Dervishi, S.M. Dunn, R. Mandal, B. Han, D.S. Wishart, B.N. Ametaj. 2017. Metabotyping reveals distinct metabolic alterations in ketotic cows and identifies early predictive serum biomarkers for the risk of disease. Metabolomics 13 43-57.

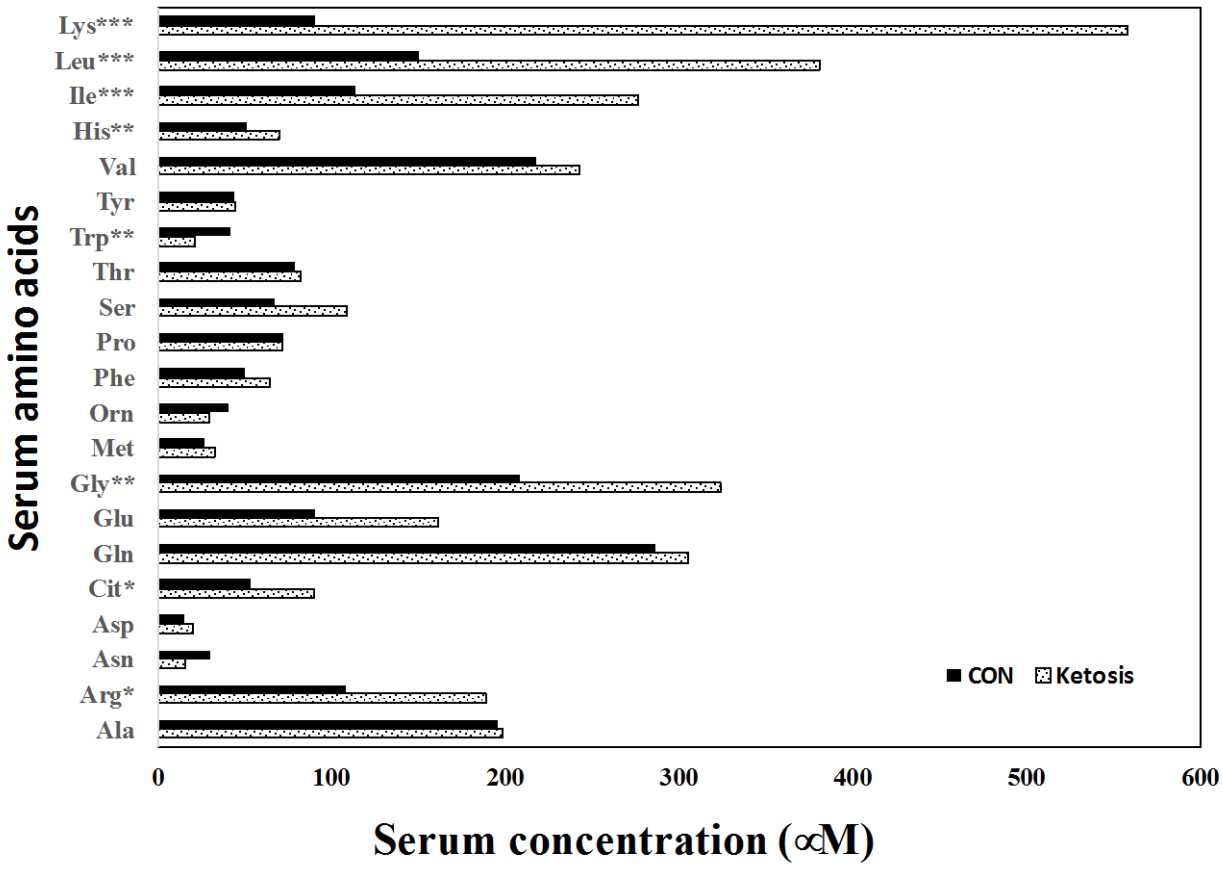


Figure 1. Amino acids in the serum of preketotic and control cows at -8 weeks prior to parturition (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

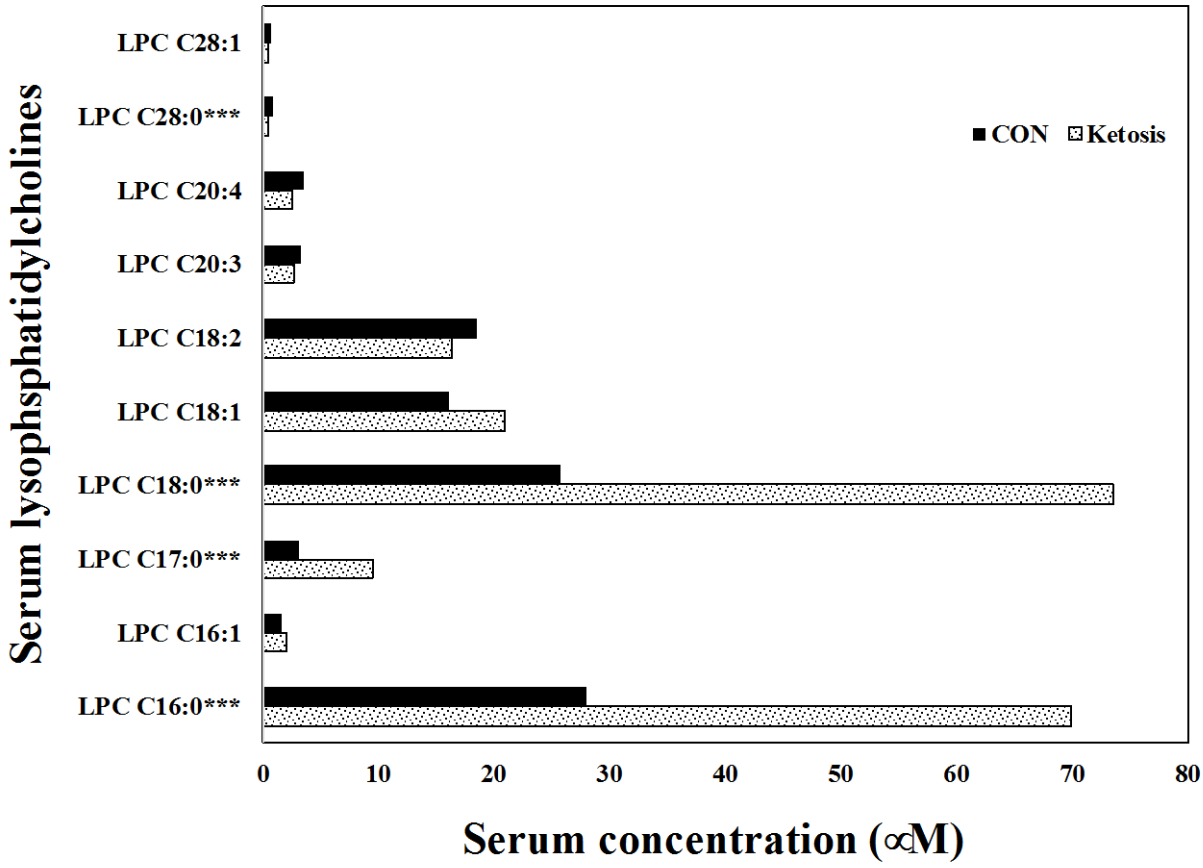


Figure 2. Lysophosphatidylcholines in the serum of preketotic and control cows at -8 weeks prior to parturition (***$P < 0.001$).

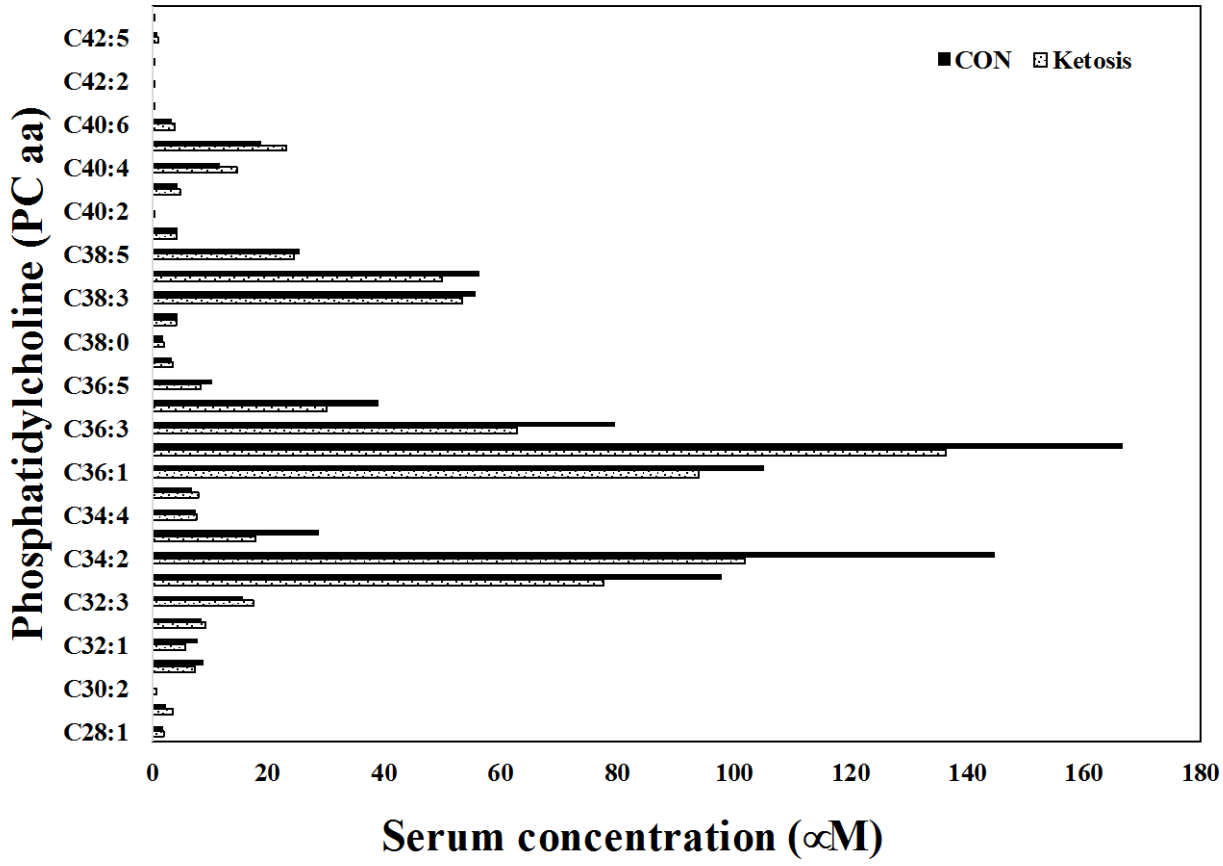


Figure 3. Phosphatidylcholines (PC aa) in the serum of preketotic and control cows at -8 weeks prior to parturition.

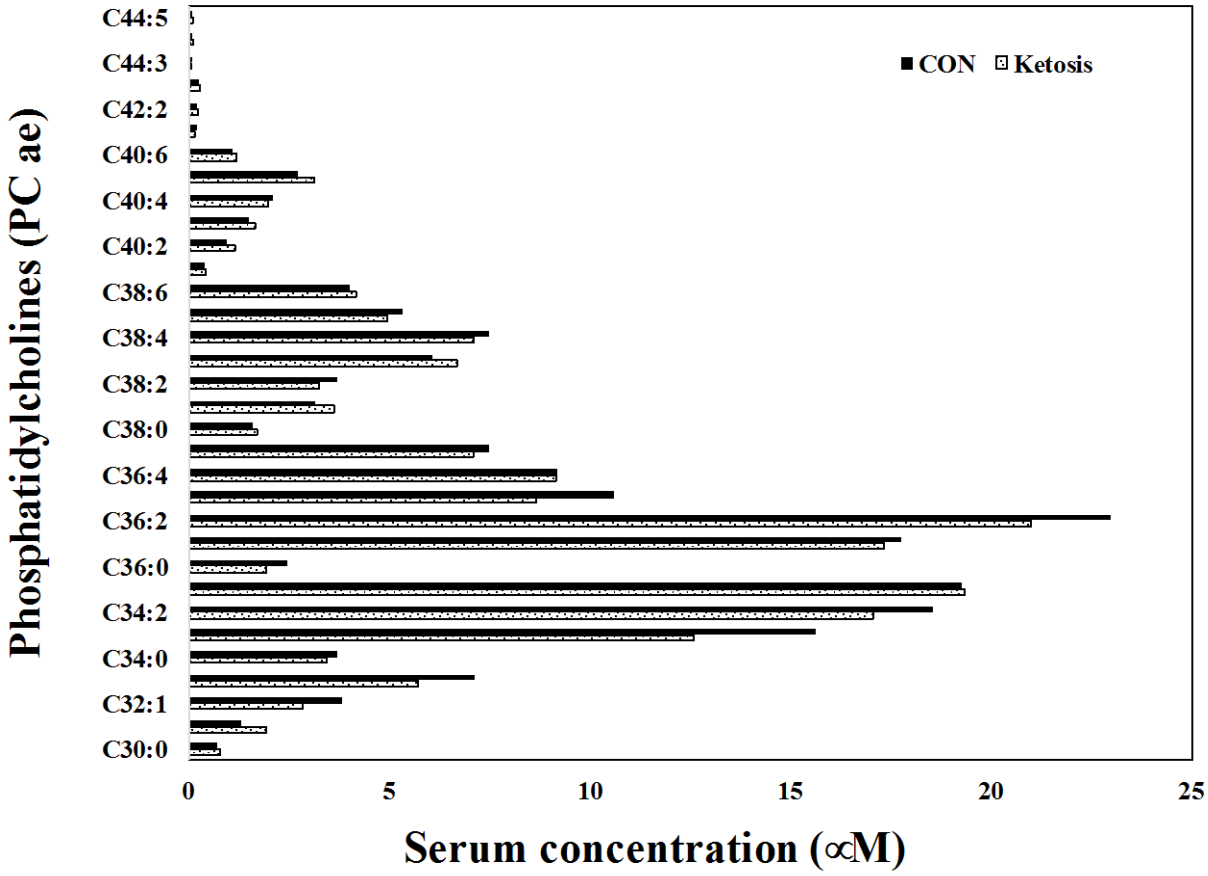


Figure 4. Phosphatidylcholines (PC ae) in the serum of preketotic and control cows at -8 weeks prior to parturition.

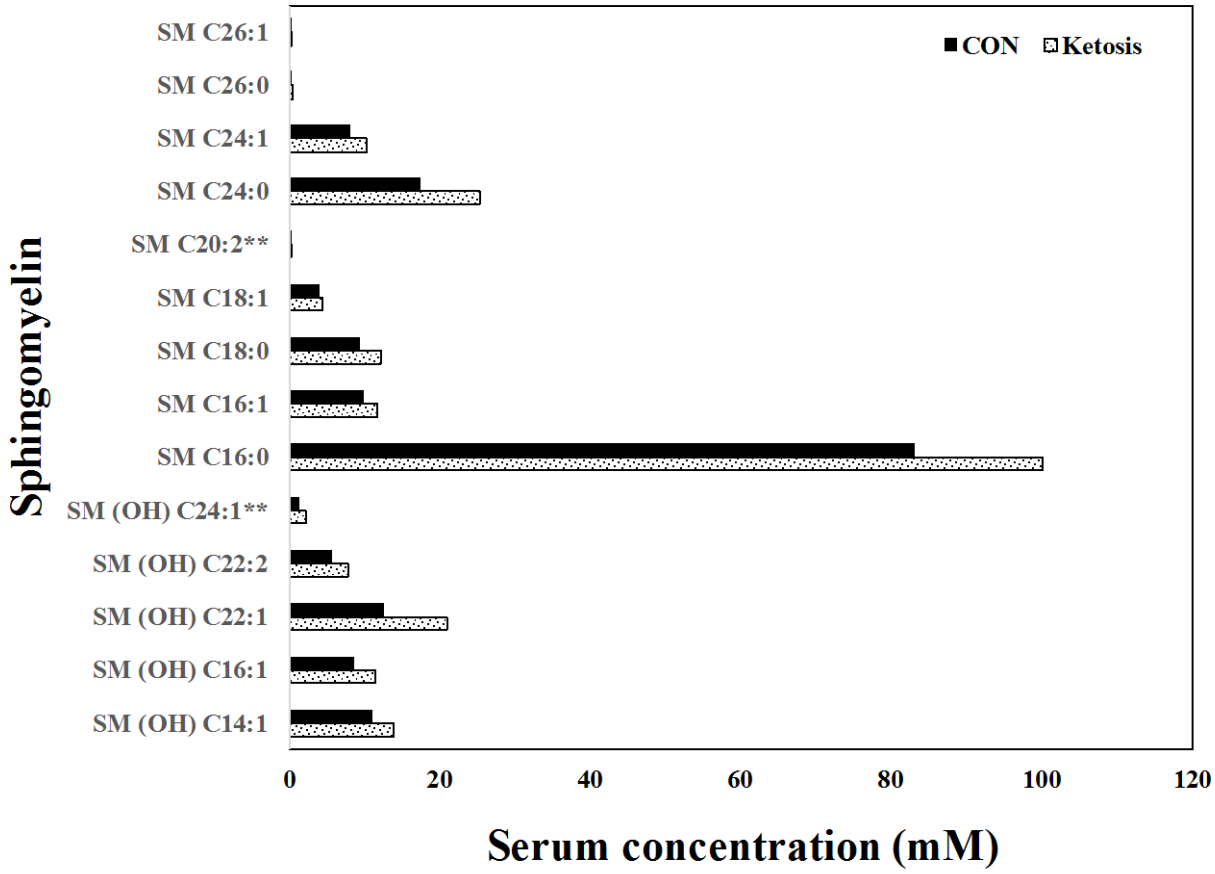


Figure 5. Sphingomyelins in the serum of preketotic and control cows at -8 weeks prior to parturition (**$P < 0.01$).

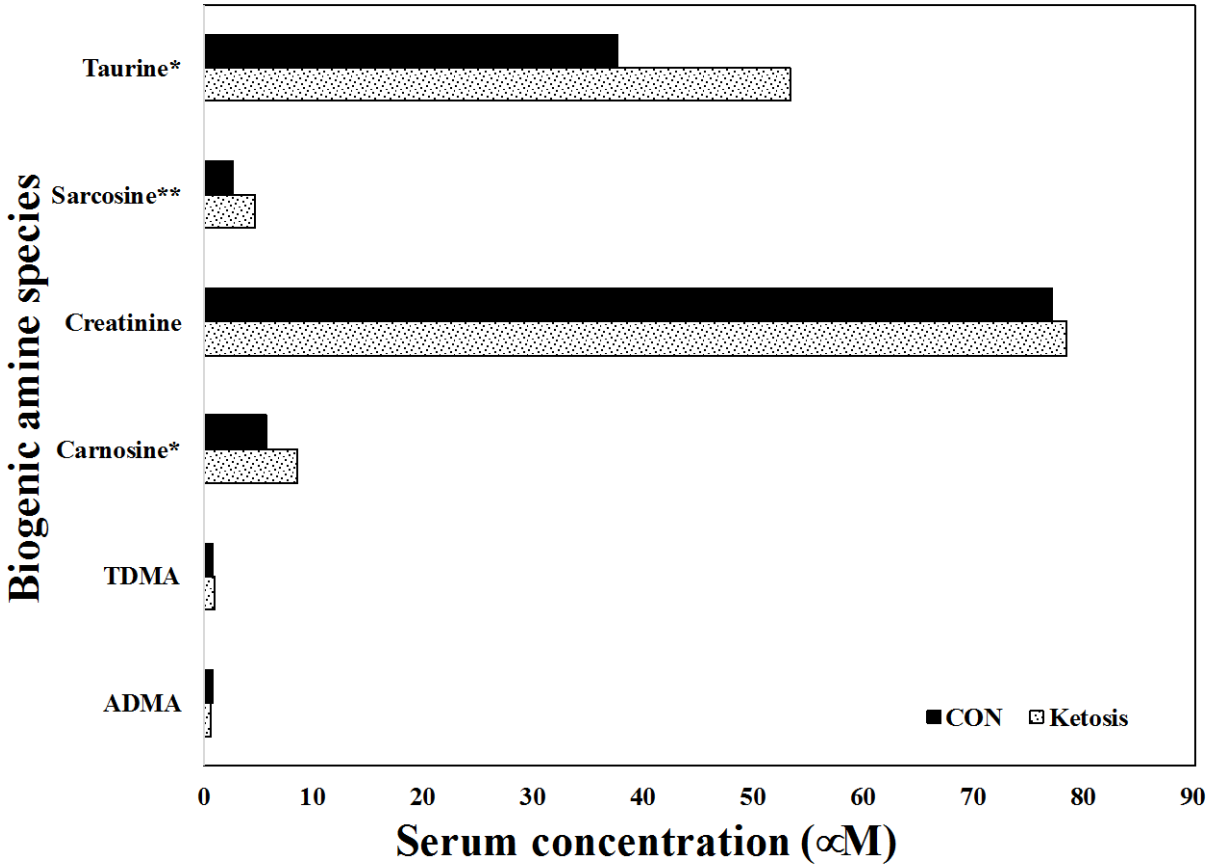


Figure 6. Biogenic amines in the serum of preketotic and control cows at -8 weeks prior to parturition (*$P < 0.05$, **$P < 0.01$).

Challenges in Identifying Viable Alternatives to Antimicrobials in Beef Cattle Production

Défis d'identification de solutions de rechange viables aux antimicrobiens en production bovine

Tim A. McAllister
Principal Research Scientist
Agriculture and Agri-Food Canada
Lethbridge Research Centre
Lethbridge, AB, T1J 4B1
tim.mcallister@agr.gc.ca

Abstract

Public scrutiny over the use of antimicrobials in beef cattle, along with the emergence of local and international markets for 'natural beef' has increased interest in identifying viable alternatives to antimicrobials. As a result, a vast array of technologies including probiotics, prebiotics, plant bioactives, immune stimulators, bacteriophage, microbial peptides and killer bacteria have been proposed as alternatives to existing antimicrobials. At this point, none of these technologies appear to control the principal infectious diseases in beef cattle as well as those antimicrobials already used to the control these diseases in Canadian beef cattle. A number of factors contribute to the challenge of developing alternatives to existing antimicrobials. Bacteria are masters of adaptation; almost inevitably developing resistance to most alternatives. The microbiomes of the gastrointestinal (GI) and respiratory tracts are exceedingly complex making it challenging for foreign bacteria to integrate and alter the composition or function of established populations. Bacteria most often reside within complex biofilms which protect members of the community from most antimicrobials. Many alternatives lack specificity equally inhibiting commensal and pathogenic bacteria or are too specific, targeting only a subpopulation of pathogenic bacteria. Immune stimulators rely on a robust immune response with bacterial pathogens developing systems to circumvent immune arsenals. Finally, the GI tract is a hostile environment where low pH and the presence of proteases or other enzymes often inactivates antimicrobials. Developments in gene editing and high-throughput sequencing may aide in overcoming the factors that currently preclude the identification of viable alternatives to antimicrobials.

Résumé

La méfiance du public à l'égard de l'utilisation des antimicrobiens en production bovine jumelée à l'émergence de marchés locaux et internationaux pour du « boeuf naturel » a stimulé l'intérêt pour l'identification de solutions de rechange aux antimicrobiens. Dans ce contexte, un large éventail de technologies, incluant probiotiques, prébiotiques, produits bioactifs d'origine

végétale, stimulateurs de l'immunité, bactériophages, peptides bactériens et bactéries tueuses, ont été proposées pour remplacer les antimicrobiens existants. Pour le moment, aucune de ces technologies ne semble pouvoir maîtriser les principales maladies infectieuses des bovins de boucherie aussi bien que le font les antimicrobiens présentement utilisés au Canada. Un certain nombre de facteurs contribuent à la difficulté de créer des solutions de remplacement aux antimicrobiens existants. Les bactéries maîtrisent l'art de l'adaptation; il est presque inévitable qu'elles acquerront une résistance à la plupart des solutions de rechange. Les microbiomes des voies digestives et respiratoires sont extrêmement complexes, de sorte qu'il est difficile pour les bactéries étrangères d'intégrer les populations établies et d'en modifier la composition ou la fonction. Le plus souvent, les bactéries vivent au sein de biofilms complexes qui protègent les membres de la communauté contre la plupart des antimicrobiens. De nombreuses solutions de remplacement manquent de spécificité et inhibent indistinctement les bactéries commensales et pathogènes ou, au contraire, sont trop spécifiques et ne visent qu'une sous-population de bactéries pathogènes. L'efficacité des stimulateurs de l'immunité repose sur de fortes réponses immunitaires contre lesquelles les bactéries pathogènes sont capables de se prémunir. Enfin, le tube digestif est un milieu hostile où le pH acide et la présence de protéases ou d'autres enzymes inactive souvent les antimicrobiens. Les progrès réalisés dans les domaines de la manipulation génétique et du séquençage à haut débit pourraient aider à contourner les difficultés qui nuisent présentement à l'identification de solutions de rechange viables aux antimicrobiens.

Introduction

The discovery of antibiotics (antimicrobials) was one of the most significant developments in contemporary science. Prior to the 1940s, infectious disease was the predominant cause of human death (Jones et al., 2012). In the current era of antimicrobial therapy, the majority of bacterial infections in both humans and animals can be successfully treated. Despite this, the effectiveness of antimicrobial therapy is threatened by the emergence of antimicrobial resistant (AMR) bacteria, and constrained by the limited number of new and novel antimicrobials in the discovery pipeline. Antimicrobial resistance is a global concern for both human and veterinary medicine. The use of antimicrobials in livestock production is contentious because of the potential for the genesis and transmission of AMR genes (ARGs) between both veterinary pathogens and zoonotic pathogens that may threaten humans. Presently, there are a myriad of alternatives to antimicrobials being investigated for their application in beef cattle production, but a number of biological challenges must be overcome if they are to achieve the same efficacy as antimicrobials that are presently used in beef cattle production. Many of the barriers that are impeding the identification of effective alternatives are the same as those that are presently diminishing the effectiveness of existing antimicrobials.

Bacteria are masters at adaptation

As with the rest of the natural world, bacteria are in a state of continuous evolution. Unlike complex organisms such as cattle or humans, bacteria have exceedingly short life cycles and entirely new generations can be generated within hours or days. Consequently, the opportunity for intergenerational evolution in bacteria is far greater than in higher life forms. Furthermore, bacteria exist in the environment in unimaginable numbers. For example, there are more bacteria

in 1 mL of rumen fluid (10 billion) than people on earth. Thus, the likelihood that one individual bacterium will express a unique genetic trait that will confer resistance to an alternative antimicrobial is far greater than with organisms that exist in far lower numbers.

Bacteria have also evolved several mechanisms of exchanging genetic material (Figure 2, Levy 1992). If the genetic material codes for a trait that confers resistance to a particular compound, then there is a high likelihood that recipient bacteria will display this same resistance. Resistance genes are exchanged via three main mechanism; conjugation, transduction and transformation (Wozniak et al. 2010). Conjugation is the process through which plasmids are exchanged between bacteria. Resistance genes are frequently carried on plasmids, which are loops of DNA that readily undergo both intra- and inter-species transfer (Flint et al. 1987). Transduction is the process whereby bacteria can become infected with viruses (i.e., bacteriophage) that pick up resistance genes and transfer them during the infection of other bacteria. Finally, transformation involves the uptake of 'free DNA' from adjacent bacteria that have died and lysed. If genes in this DNA code for resistance to antimicrobial alternatives, bacteria that acquire this DNA can become resistant. Resistance genes, acquired through conjugation, transduction, or transformation must integrate and remain sufficiently stable in the bacterial cell so as to allow their expression and the generation of the protein products that confer resistance. In many cases whole segments of DNA have specialized properties that promote chromosomal integration, often introducing whole families of resistant genes in a single transfer event (Bass et al. 1999).

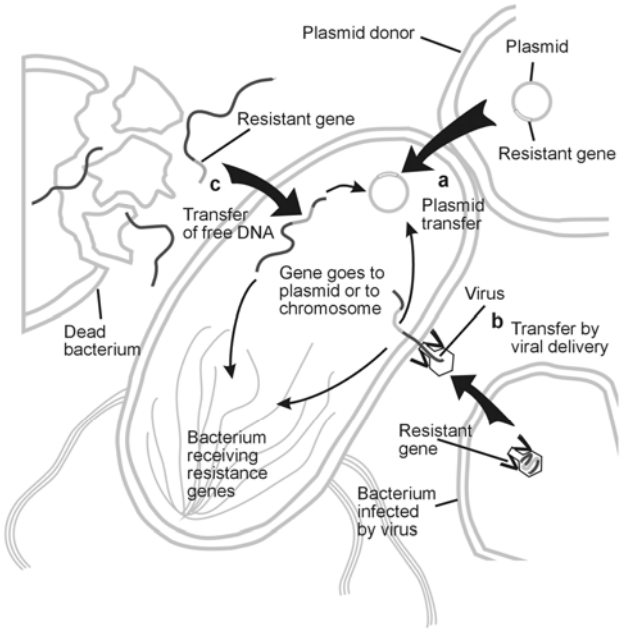


Figure 1. Mechanisms of gene transfer in bacteria a) transfer of plasmid from another bacterial cell; b) transfer via viral carrier; c) uptake of free DNA. Acquired DNA can carry a variety of genes encoding for resistance to alternatives to antimicrobials.

Integrative conjugative elements (ICEs) are a form of mobile genetic element (MGE) that have gained much interest in the last couple of years. Unlike other MGEs, ICEs are self-transmissible as they encode all the machinery required for them to excise from the chromosome, circularize and replicate into a new host through conjugation (Wozniak et al. 2010). ICEs have been

identified in both gram-positive and gram-negative bacteria, with many occupying a wide host range (Wozniak et al. 2010). ICE can carry genes coding for resistance against a variety of factors that could threaten the survival of bacteria. This could include resistance to biocides, plant bioactives, metals and existing antimicrobials. For example our lab isolated bacteria that cause pneumonia in cattle that were resistant to 11 different antibiotics (Klima et al. 2014). The ability of ICE to carry multiple resistance genes and transfer to a wide host range makes them an important vehicle in horizontal gene transfer (HGT).

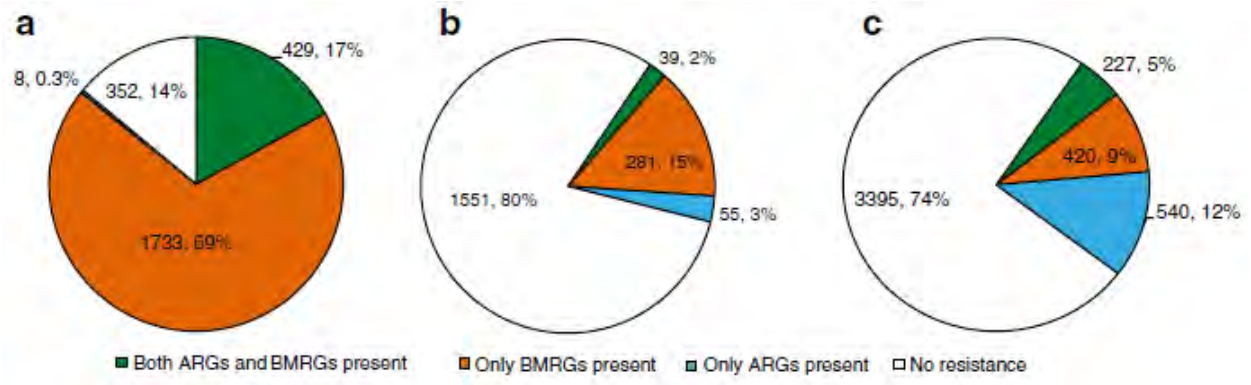


Figure 2. Antimicrobial resistant genes (ARGs) and biocide and metal resistant genes (BMRGs) in (a) 2522 bacterial genomes; (b) 1926 plasmids within these genomes and (c) 4582 plasmids . Resistance to both ARGs and BMRGs was more common when BMRGs were associated with either the genome or plasmid (Pal et al. 2015).

The fact that genes coding for resistance to a variety of environmental challenges tend to travel together, raises the possibility that resistance to alternatives to antimicrobials can arise from selective pressures other than the alternative itself. For example, genes that alter cell membrane permeability may change the sensitivity of the bacterial cell to plant bioactives, microbial peptides or even bacteriophage. Such an occurrence would make it exceedingly difficult to ensure the efficacy of alternatives to antimicrobials over a range of production conditions.

Bacterial communities are incredibly stable and do not readily accept outsiders.

Only recently has the true complexity of the cattle microbiome been realized, which is estimated to contain 10 times the number of cells that make up the host animal. Furthermore, the majority (> 80%) of these microbes have never been cultured in the laboratory and consequently little is known about their function or role within the microbial community. Members of the microbiome interact in an integrated fashion often coordinating activities through cell to cell communication mechanisms such as quorum sensing. The shear density of microbes in the respiratory and GI tracts also creates a highly competitive environment in which available substrates are rapidly utilized by members of the community, frequently in a synergistic manner. Consequently, probiotics that are introduced into these environments often fail to thrive as they cannot speak the 'bacterial language' or lack the biochemical machinery needed to survive (Figure 3). Chances of establishment of probiotics may be greater if they produce antimicrobials that specifically exclude competitors. However, even under these conditions establishment can be difficult for the reasons described herein. Integration of probiotics into the microbiome may be more realistic if they possess unique metabolic activities that promote their

establishment within the intestinal tract, such as the ability of *Synergistes jonesii* to degrade the toxin, mimosine in the rumen (Jones et al. 2009). Integration of probiotics into the microbiome so as to carry out more complex functions such as the degradation of plant cells seems less likely, especially as most probiotics lack genes coding for the enzymes needed to digest plant cell walls. At this point it appears that probiotics may have the greatest potential in young cattle where the microbiome has yet to become fully established.

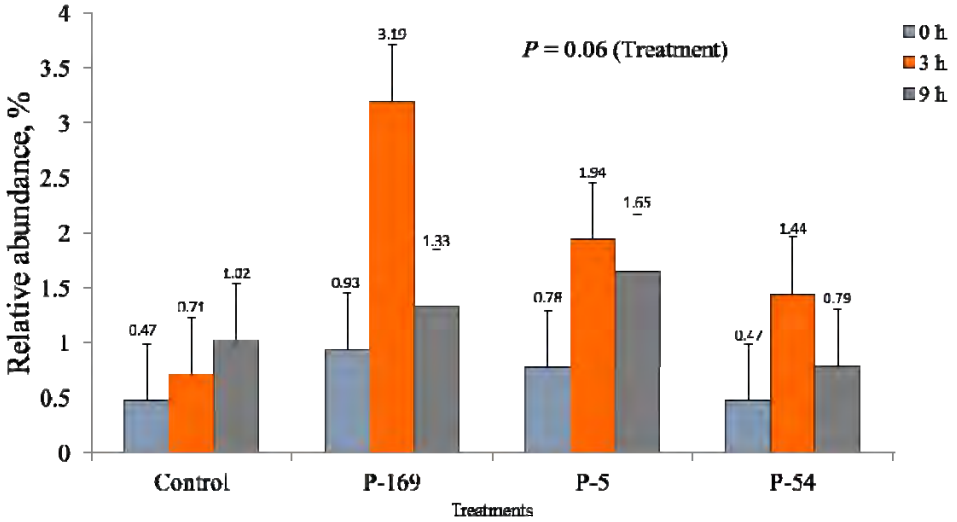


Figure 3. Relative abundance of various probiotic strains of propionibacterium introduced into the rumen at 5 x 10^9 after 0, 3 and 9 h in cattle fed a high forage diet (Viyas et al. 2014). Note that although relative abundance of propioniobacterium was higher 3 h after inoculation, they returned to pretreatment levels within 9 h.

Biofilms are resilient

Bacteria grown in liquid cultures typically grow planktonically as individual cells that are susceptible to antimicrobial agents. However, within natural environments such as in the gastrointestinal and respiratory tracts, bacteria form complex biofilm communities that are densely bound by extracellular polymeric substances. Bacteria growing in biofilms can exhibit up to a thousand-fold greater resistance to antimicrobials as compared to their planktonic counterparts. Factors such as physical exclusion, slow growth rates and expression of enzymes that degrade antimicrobials can render alternatives to antimicrobials ineffective (Gabrani et al., 2015). Patterns of gene expression are often altered in biofilms, rendering alternatives that were developed to kill planktonic cells ineffective. Biofilms can act as a reservoir of these resistant bacteria that are continuously shed into the environment where they can establish new resistant communities. Biofilm can also harbor 'persisters' which are bacteria that essentially shut down their metabolism, creating a situation where alternatives are ineffective as the metabolic pathways that they target are inactive. Biofilms may also increase the rate of mutations within its members, accelerating the ability of the community to adapt to environmental challenges posed by alternatives to antimicrobials. Genomic approaches that improve the understanding of biofilm complexity and community composition (Sauer, 2003), may aide in the identification of therapeutic agents that degrade (Gökçen et al., 2014) or inhibit the formation of biofilms

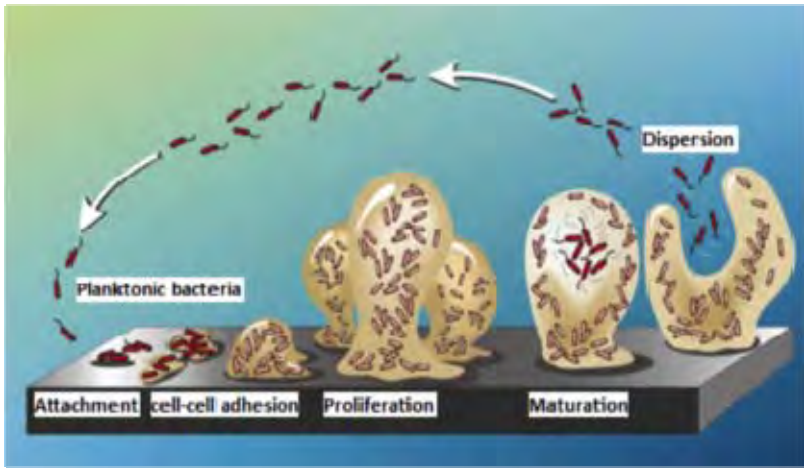


Figure 4. Biofilms are formed as planktonic bacteria attach to surfaces and form complex communities that resist alternatives to antimicrobials. This can be achieved through physical exclusion, changes in gene expression, heightened rates of adaptive mutations or the formation of 'persister' cells that are unresponsive to alternatives. Cells can also disperse from the biofilm and establish new communities.
http://www2.le.ac.uk/projects/vgec/schoolscolleges/topics/microbial-genetics-1/infection/biofilms

Gastrointestinal tract is a hostile place.

The microbial population within the GI tract represents a unique ecosystem with the ability to degrade a myriad of substrates. This accounts for the ability of ruminants to consume higher levels of mycotoxin contaminated feed as compared to swine and poultry. This same degradative capacity can also be directed towards alternatives to antimicrobials. Concentrations of soluble carbohydrates and proteins in the rumen are kept low as both are rapidly degraded by endemic carbohydrases and proteases, respectively. Consequently, alternatives to antimicrobials (e.g., antibodies, bacteriocins, carbohydrate-based prebiotics) that contain these compounds are frequently degraded before they have an opportunity to act on the intestinal microbiome. Many of the more chemically complex plant bioactives may have short term impacts, but these effects are often lost as the microbial population adapts and degrades them after a period of days or weeks. Additives are also exposed to extremes in pH (2.0 to 7.0) as they travel through the digestive tract, often not remaining active over this range. Finally, natural members of the microbiome can produce lysogenic substances that kill probiotics and prevent them from integrating into the microbiome. Alternatives to antimicrobials that are not able to cope with this hostile environment are unlikely to be efficacious.

Conclusions

There is an urgent need to develop alternatives to the antimicrobials that are presently used in beef cattle production. Part of this need is driven by the desire of consumers to see subtherapeutic antimicrobials eliminated from beef cattle production systems. Perhaps even more importantly, bacteria are exhibiting high levels of resistance to many of the antimicrobials currently used, reducing their effectiveness for controlling infectious diseases in cattle. Many of the barriers that are preventing the identification of viable alternatives are the same as those that are undermining the value of antimicrobials presently in use. Some alternatives are too specific (e.g., bacteriophage) targeting only a fraction of pathogenic populations, whereas others (e.g.,

plant bioactives) are too broad, impacting both pathogenic and commensal bacteria. To believe that bacteria cannot become resistant to new alternatives to antimicrobials is a demonstration of naivety. Development of viable alternatives to existing antimicrobials will only be achieved through an appreciation for the complexity and adaptive ability of microbial populations.

References

Bass, L., Liebert, C.A., Lee, M.D., Summers, A.O., White, D.G., Thayer, S.G. and J. J. Maurer. 1999. Incidence and characterization of integrons, genetic elements mediating multiple-drug resistance, in avian *Escherichia coli*. Antimicrob. Agent Chemo. 43:2925-2929.

Gabrani, R., Sharma, G., Dang, S. and S, Gupta. 2015. Interplay among bacterial resistance, biofilm formation and oxidative stress for nosocomial infections. In Free Radicals in Human Health and Disease (pp. 369–379). http://doi.org/10.1007/978-81-322-2035-0_23

Gökçen, A., Vilcinskas, A. and J. Wiesner. 2014. Biofilm-degrading enzymes from Lysobacter gummosus. Virulence. **5**: 378–387. http://doi.org/10.4161/viru.27919

Flint, H.J., Duncan, S.H. and C. S. Stewar. 1987 Transmissible antibiotic resistance in strains of *Escherichia coli* isolated from the bovine rumen. Lett. Appl. Microbiol. 5:47-49.

Jones, R. J., Coates, D. B. and B. Palmer. 2009. Survival of the rumen bacterium *Synergistes jonesii* in a herd of droughtmaster cattle in north Queensland. Anim. Prod. Sci. **48**: 643-645.

Jones, D. S., Podolsky, S. H. and J. A. Greene. 2012. The Burden of Disease and the Changing Task of Medicine. New Eng. J. Med. **366**: 2333–2338. http://doi.org/10.1056/NEJMp1113569

Klima, C.L., R. Zaheer, S.R. Cook, C.W. Booker, S. Hendrick, T.W. Alexander and T.A. McAllister. 2014. Pathogens of bovine respiratory disease in North American feedlots conferring multidrug resistance via integrative conjugative elements. J. Clin. Microbiol. 52:438-448.

Levy, S.B. 1992. The antibiotic paradox. How miracle drugs are destroying the miracle. Plenum Press, New York, NY.

Pal, C, Bengtsson-Palme, J., Kristiansson, E. and D. G. Joakim Larson. 2015. Co-occurrence of resistance genes to antibiotics, biocides and metals reveals novel insights into their co-selection potential. BMC Genomics 16: 964.

Sauer, K. 2003. The genomics and proteomics of biofilm formation. Genome Biol. 4: 219. http://doi.org/10.1186/gb-2003-4-6-219

Vyas, D., McGeough, E. J., Mohammed, R., McGinn, S. M., McAllister, T. A. and K. A. Beauchemin. 2014. Effects of Propionibacterium strains on ruminal fermentation, nutrient digestibility and methane emissions in beef cattle fed a corn grain finishing diet. Animal 8: 1807-1815.

Wozniak, R.A.F. and M.K. Waldor. 2010. Integrative and conjugative elements: mosaic mobile genetic elements enabling dynamic lateral gene flow. Nat. Rev. Microbiol. 8:552-563.

Evaluating the Variability of Transfer of Passive Immunity in Quebec Dairy Herds

Évaluation de la variabilité du transfert d'immunité passive dans les troupeaux laitiers du Québec

M-P.Morin, P.Freycon, S.Buczinski , J.Dubuc
Département des Sciences Cliniques, Faculté de Médecine Vétérinaire
Université de Montréal, St-Hyacinthe, J2S 7C6

Abstract

This study quantified the prevalence of success of passive immunity transfer (SPIT) in dairy herds and investigated its association with colostrum management at the herd-level. Local dairy herds were asked to collect first colostrum meal samples (n = 20 calves/herd) from which IgG concentration was derived using a Brix refractometer. Bacterial contamination of colostrum was quantified using standard bacteriology laboratory plate count. Adequate aerobic contamination (ADEQAERO) was defined as < 100,000 cfu/ml. The volume of colostrum fed and its IgG concentration were combined to estimate the amount of IgG ingested by calves (sufficient amount ≥ 150g IgG; SAIGG). Ingestion of first colostrum meal within the first 6 hours of life was considered adequate (ING6H). A jugular blood sample was obtained from calves at 1-7 days of age to assess SPIT (defined as serum ≥8.4 % Brix). The preliminary results are based on 333 calves from 18 commercial herds. The median herd-level SPIT and prevalence of SAIGG ranged from 41 to 100% (median: 70%) and from 18% to 100% (median: 66%), respectively. The median herd-level prevalence of ING6H and ADEQAERO ranged from 47% to 96% (median: 77%) and from 3% to 75% (median: 38%), respectively. Adequate timing, amount of IgG and bacterial contamination were associated with greater SPIT prevalence.

Résumé

Cette étude a permis de quantifier la prévalence de succès du transfert d'immunité passive (SPIT) dans les troupeaux laitiers et d'étudier les liens entre le SPIT et la gestion du colostrum à l'échelle du troupeau. On a demandé à des producteurs laitiers locaux de prélever des échantillons de premier repas de colostrum (n = 20 veaux par troupeau) dans lesquels la concentration d'IgG a été mesurée à l'aide d'un réfractomètre Brix. La contamination bactérienne du colostrum a été quantifiée par procédé standard de comptage sur plaque. Le niveau de contamination adéquat par les microorganismes aérobies (ADEQAERO) a été fixé à < 100 000 ufc/mL. Les valeurs de volume de colostrum servi et de concentration d'IgG ont été combinées pour évaluer la quantité d'IgG ingérée par les veaux (quantité suffisante ≥ 150 g IgG; SAIGG). Un premier repas de colostrum pris dans les 6 heures suivant la naissance a été considéré comme adéquat (ING6H). Un échantillon de sang recueilli au niveau de la jugulaire a été prélevé chez les veaux âgés de 1 à 7

jours pour mesurer le SPIT (fixé à une concentration sérique ≥ 8,4 % Brix). Les résultats préliminaires sont basés sur l'étude de 333 veaux provenant de 18 troupeaux commerciaux. Les valeurs médianes de SPIT et de prévalence de SAIGG à l'échelle du troupeau ont oscillé entre 41 % et 100 % (médiane : 70 %) et entre 18 % et 100 % (médiane : 66 %), respectivement. Les valeurs médianes de prévalence de ING6H et de ADEQAERO à l'échelle du troupeau ont oscillé entre 47 % et 96 % (médiane : 77 %) et entre 3 % et 75 % (médiane : 38 %), respectivement. Les valeurs adéquates de délai avant consommation, de quantité d'IgG et de contamination bactérienne ont été associées à une prévalence de SPIT plus élevée.

Leaky gut's contribution to inefficient nutrient partitioning

Lance Baumgard, Sara Stoakes and Rob Rhoads
Iowa State University

Department of Animal Science

General Synopsis

Heat stress → Reduced Feed Intake
Heat stress → Leaky Gut
Leaky Gut → Activated Immune System
Activated Immune System → Nutrient Reprioritization to non-productive functions
Nutrient Reprioritization to non-productive functions → Reduced Performance

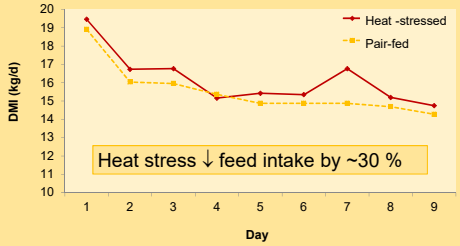
Heat Stress Questions??

- Does the decrease in feed intake explain the reduced milk yield during heat stress?

  Indirect vs. direct effects of heat

- If we have a better understanding of the biological reasons WHY heat stress reduces production, we'll have a better idea of how to alleviate it.

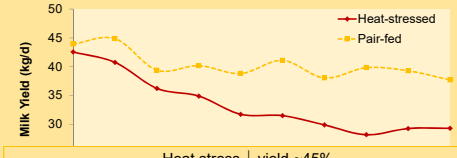
Lactation: Effects of Heat Stress on Feed Intake



Heat stress ↓ feed intake by ~30 %

Rhoads et al., 2009

Effects of Heat Stress on Milk Yield

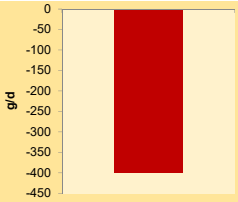


Heat stress ↓ yield ~45%
Pair-feeding ↓ yield by ~19%

Thus, ↓ feed intake only accounts for ~50% of the reductions in milk yield

Wheelock et al., 2010
Baumgard et al., 2011

Milk Sugar Output



Heat Stress Cows Secrete ~400 g less lactose/day than Pair-Fed Thermal Neutral Controls

Is the liver producing ~ 400 g less glucose/day??
or is extra-mammary tissues utilizing ~400 g more/day?

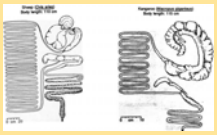
Rhoads et al., 2009
Wheelock et al., 2010

Gastro-Intestinal Tract (GIT) Review

Guts

Reminder: Intestinal Functions

- GIT is a tube running from the mouth to the anus
  - Everything inside of the tube is technically "outside" of the body
- Digest and absorb nutrients
  - GIT lumen is a inhospitable environment
- Prevent parasites, pathogens, enzymes, acids, toxins etc.. From infiltrating "self"
  - Barrier function



Human GIT Surface Area:

That's an enormous amount of area to "defend"!
No wonder 70% of the immune system resides in GIT

Biology of Heat Stress Symptoms

Heat Stress and Gut Health

- Diversion of blood flow to skin and extremities
- Coordinated vasoconstriction in intestinal tissues
  - Reduced nutrient and oxygen delivery to enterocytes
  - Hypoxia increases reactive oxygen species (ROS)
- Reduced nutrient uptake increases rumen and intestinal osmolarity in the intestinal lumen
  - Multiple reasons for increased osmotic stress

Baumgard and Rhoads, 2013

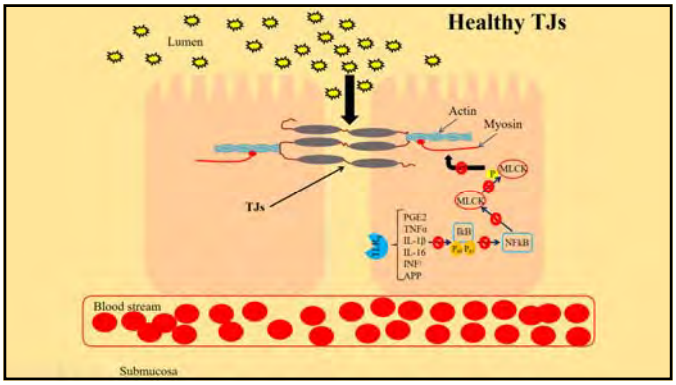
Intestinal Morphology

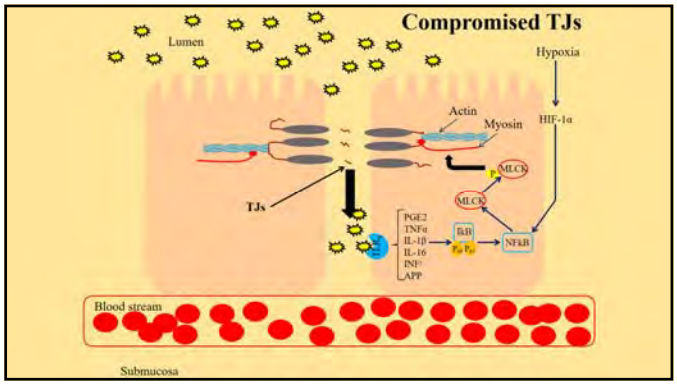
Thermal Neutral | Heat Stress | Pair-fed

Pearce et al., 2011

Heat Stress and Gut Health

- Lipopolysaccharide (LPS) stimulates the immune system
- LPS promotes inflammation production….catabolic condition
  - TNFα, IL-1 etc..
    - Reduced appetite
    - Stimulates fever
    - Causes muscle breakdown
    - Induces lethargy
    - ….reduces productivity
- LPS can cause liver damage





Heat Stress Summary

- Direct and indirect effects
  - ↓DMI only accounts for 50% of reduced milk yield
- Hyperinsulinemia
- Blunted adipose mobilization
- Liver remains sensitive to catabolic signals
- Leaky gut
  - Inflammation and acute phase protein response
- Unknown whereabouts of 400 g of glucose

Leaky Gut and Ketosis?

Dogma

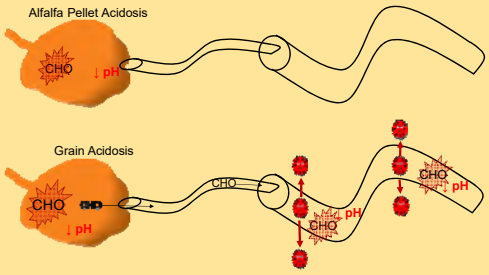
- Excess adipose tissue mobilization causes fatty liver and ketosis
- This is worse in high producing cows
- Industry Goal: Reduce blood NEFA

Correlation Studies

- Many studies *associate* NEFA and BHBA with:
  - Increased risk of ketosis, decreased milk yield, LDA, metritis, retained placenta, laminitis, or poor reproduction
    - Chapinal et al., 2011; Huzzey et al., 2011; Ospina et al., 2010a, 2010c; Duffield et al., 2009; LeBlanc et al., 2005
  - Plasma NEFA are markedly increased (>700 mEq/L) following calving in almost all cows
    - ~15-20% get clinical ketosis
    - What makes these cows more susceptible to ketosis?
      - Predisposition to developing fatty liver?

Interesting Observations?

- Incidence of clinical ketosis in Southwest vs Midwest and Northeast
  - ~0.5% vs. 10-15%
- Heat Stress cows have increased incidence of fatty liver
- Rumen acidosis:
  - Ground grain: systemic inflammation
  - Alfalfa pellets: no inflammation



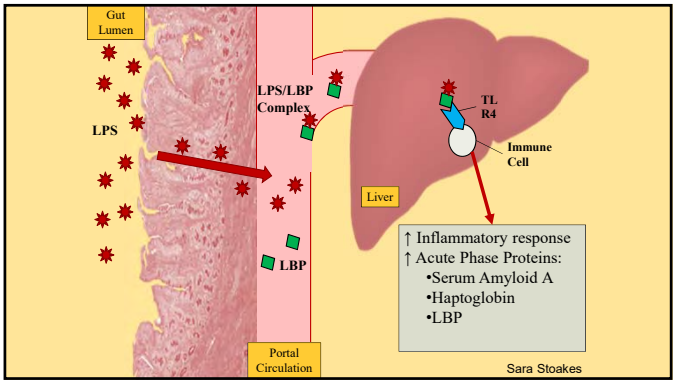
Cartoon created from comments made within Dr. Kees Plazier's papers

Transition Period & Inflammation

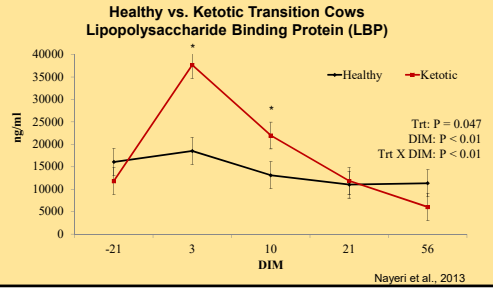
- Associated with or partly responsible for transition period issues?
  - Hailemariam et al., 2014
- Homeorhetic adaptation to lactation?
  - Farney et al., 2013
- Inflammation source???
  - General (sterile inflammation)
  - Uterus
  - Mammary
  - Gastrointestinal tract?

Leaky Gut and Liver NEFA Intolerance?

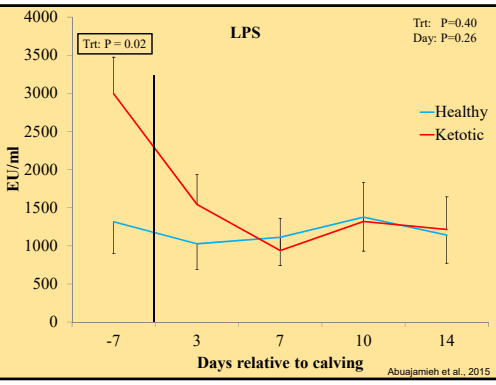
- Reasons for increased likelihood of leaky gut in transition dairy cows???
  - Dietary shift to increased concentrates
    - Rumen acidosis with grain compromises GIT barrier and increases blood LPS
      - Starch delivery to the hind-gut
    - Rumen acidosis with alfalfa pellets does not increase blood LPS
      - Plaizier et al., 2012; 2009
  - Distally derived cytokines
    - Paibomesai et al., 2013



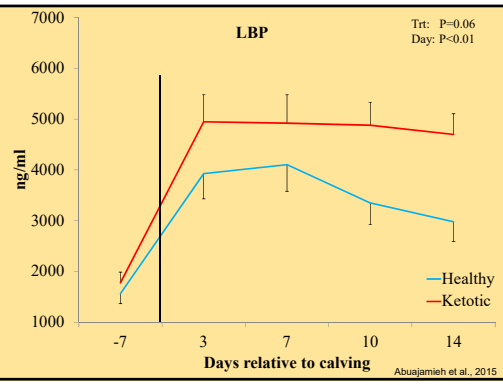
Increased LBP in Ketotic Cows



Nayeri et al., 2013



Abuajamieh et al., 2015



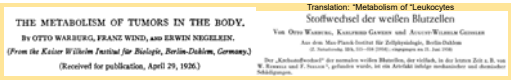
Abuajamieh et al., 2015

Transition Cow Problems are Associated with Biomarkers of Leaky Gut

- Intestinal barrier becomes leaky
- Endotoxin induced immune activation
- Immune system has energetic cost
- Reprioritization of nutrients away from milk synthesis……..$$$ problem

Evolution of the Immunometabolic Field

Otto Warburg



THE METABOLISM OF TUMORS IN THE BODY.

By OTTO WARBURG, FRANZ WIND, and ERWIN NEGELEIN.

(From the Kaiser Wilhelm Institut für Biologie, Berlin-Dahlem, Germany.)

(Received for publication, April 29, 1926.)

Translation: "Metabolism of "Leukocytes

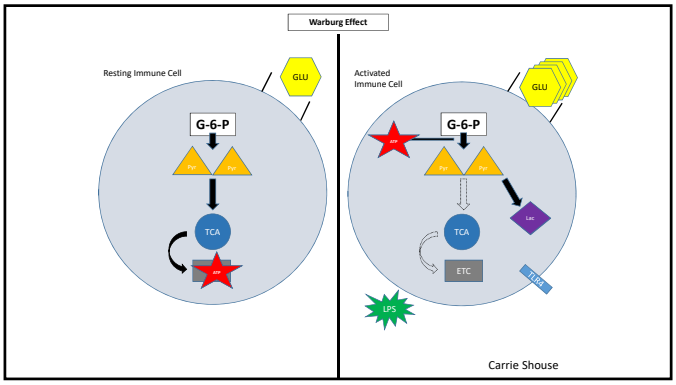
Stoffwechsel der weißen Blutzellen

- First recognized the unique metabolism of cancer cells (1927)
  - Large glucose consumers
  - Switch from oxidative phosphorylation → aerobic glycolysis
- Also observed activated lymphocytes become highly glycolytic (1958)
- Mentored Hans Krebs
- Drinking buddy with Albert Einstein

This is a picture of Otto Warburg's 1921 grant proposal.

Translation: "I require 10,000 marks"
(equivalent today of ~$75,000)

Funded!



How much glucose is the entire body using??

60 years later and we still not know how much glucose the immune system needs *in vivo*?
Prerequisite for developing mitigation strategies

What's the Problem?:

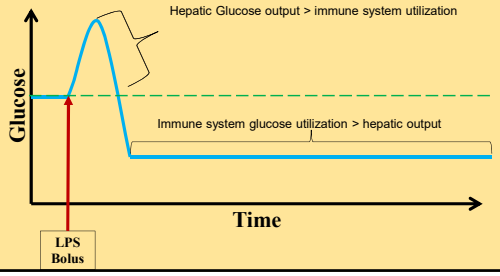
- Dynamic and ubiquitous distribution of the immune system throughout tissues
  - Allows for quasi tissue/organ quantification but….
  - Complicates whole-body quantification

Etiology of Our Hypothesis

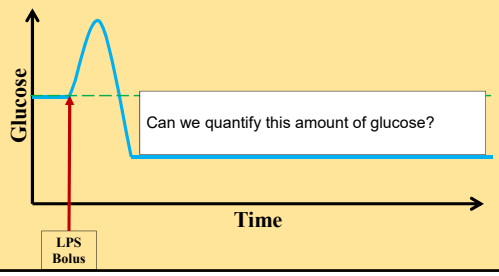
- All leukocytes become obligate glucose utilizers
- Adipocytes, myocytes and hepatocytes reduce glucose utilization
- Liver glucose output increases
- ……but hypoglycemia still occurs

Hmmmmmm????????

LPS Challenge & Blood Glucose



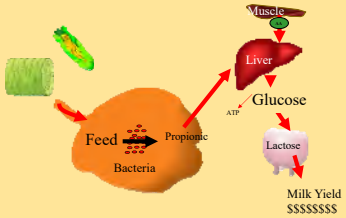
LPS Challenge & Blood Glucose



Glucose and the Immune System

- At rest, immune cells can oxidize multiple fuels
- Once activated, immune cells become obligate glucose utilizers
- How much glucose does the immune system use?
- Milk synthesis is regulated by lactose synthesis….glucose is precursor to lactose

Glucose is made from propionate
Lactose is made from glucose
Milk yield is determined by the amount of synthesized lactose



Glucose is made from propionate
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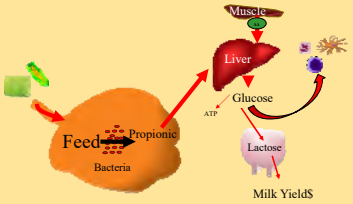
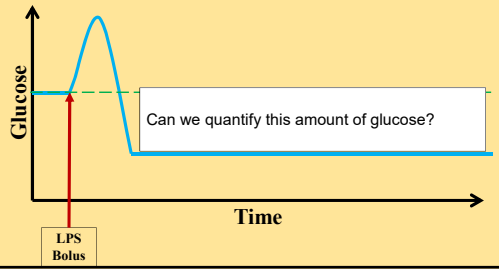
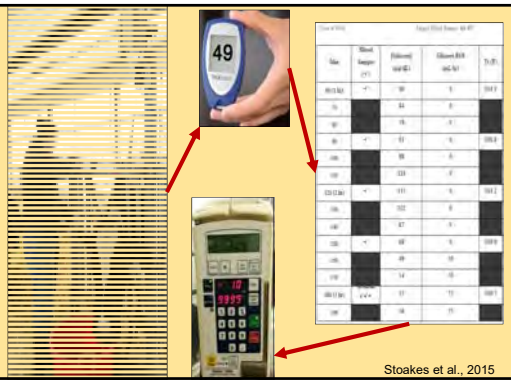


Glucose is $$

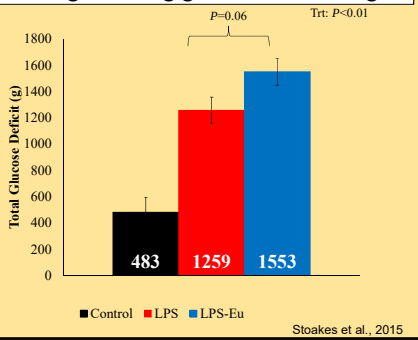
LPS Challenge & Blood Glucose





Stoakes et al., 2015

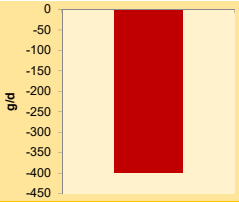
1553 g – 483 g = 1070 g glucose/12h… 2k g/d



Stoakes et al., 2015

8.4 Mcal of energy!

Remember??: Milk Sugar Output



Heat Stress Cows Secrete ~400 g less lactose/day than Pair-Fed Thermal Neutral Controls

Is the liver producing ~ 400 g less glucose/day??
or is extra-mammary tissues utilizing ~400 g more/day?

Rhoads et al., 2009
Wheelock et al., 2010

Can "leaky gut" explain suboptimal production frequently observed in animal agriculture?

- Heat Stress
- Inadequate feed intake
  - "off-feed event"
    - The negative effects on growth and milk yield are bioenergetically unexplainable by reduced feed intake
  - Transition period
    - Cause of ketosis?
  - Weaning
  - Shipping
  - Overcrowding
  - Unpalatable feed

Target Mitigation Strategies

- Encourage feed intake
- Prevent infections (no brainer)
- Maximize digestion prior to large intestine
- Prevent rumen acidosis
- Decrease intestinal permeability
- Immunomodulation
- Minimize psychological stress
- Safely maximize glucose production

$$ Billion Dollar Question $$

- Can the Feed or Animal Health Industry do anything about leaky gut????
- Targets:
  - Direct action at intestine
  - Indirect via:
    - Increased feed intake
    - Rumen acidosis prevention
      - Hind gut acidosis prevention
    - Improved immune function

Potential nutritional strategies to ameliorate intestinal permeability

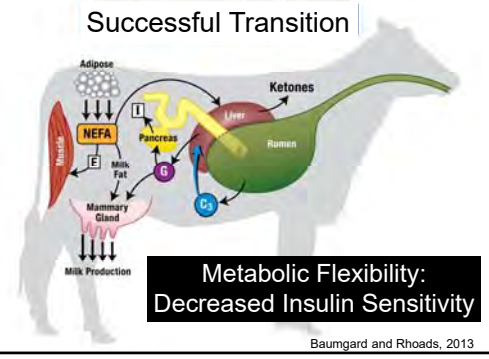
Supplement	Presumed Mechanism of Action
Bicarbonate	Acidosis prevention
Glutamine	↑ intestine integrity
Zinc	↑ intestine integrity, antioxidant
Dairy Products	↑ intestine integrity
Vitamin A	Antioxidant
Vitamin C	Antioxidant
Vitamin E	Antioxidant
Selenium	Antioxidant
Dexamethasone	↑ intestine integrity
Betaine	Osmotic regulation; CH_3 donor
Conjugated Linoleic Acid	↑ Energy balance
Chromium	↑ Feed Intake, Increase neutrophil #
Yeast, yeast extract/DFM	Acidosis prevention & ↑ Feed Intake
Ionophores	Acidosis prevention
β-glucan	Immune modulation
Mannanoligosaccharide	↑ intestine integrity
Rehydration therapy	↑ intestine integrity & ↑ Feed Intake
Butyrate	↑ intestine integrity
Mycotoxin binders	↑ intestine integrity
OmniGen-AF	Immune modulation

Baumgard et al., 2014

Lactating Dairy Cow: Consequences of Leaky Gut

Summary

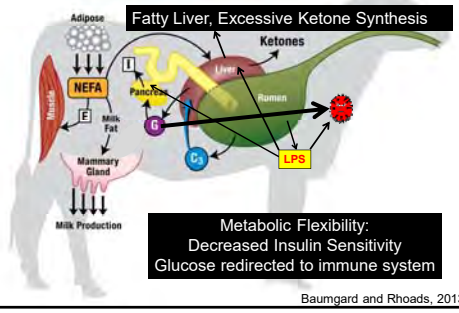
Successful Transition



Metabolic Flexibility:
Decreased Insulin Sensitivity

Baumgard and Rhoads, 2013

Unsuccessful Transition



Metabolic Flexibility:
Decreased Insulin Sensitivity
Glucose redirected to immune system

Baumgard and Rhoads, 2013

Summary

- Heat stress and ketotic cows have a similar metabolic and endocrine fingerprint
  - Leaky gut is a common denominator in both
- The activated immune system utilizes an enormous amount of glucose.
- Dietary Strategies to minimize immune activation

Conclusions

- Leaky gut may play an important role in suboptimal milk yield commonly observed during "stress"
- Strategies that can improve intestinal integrity need to be researched…in a "stressed model"
- If leaky gut is the fundamental cause of many typical on-farm problems….then it is a financial problem that dwarfs all others combined

Acknowledgments

Funding Support

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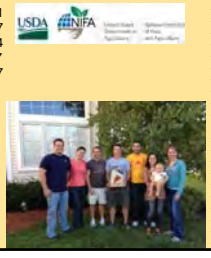
- Kemin Inc.



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TRADITION OF
EXCELLENCE
IOWA STATE UNIVERSITY

Impact of Transition Health Status of Dairy Cows on Reproductive Performance: Looking at Potential Nutritional Tools

Impact de l'état de santé des vaches laitières en transition sur la performance reproductrice : recherche d'outils nutritionnels

R.L.A. Cerri[1], E. Ribeiro[2], J.L.M. Vasconcelos[3],
[1]Applied Animal Biology, Faculty of Land and Food Systems, University of British Columbia
[2]Department of Animal Biosciences, University of Guelph
[3]Department of Animal Production, Faculty of Veterinary Medicine and Animal Science, Sao Paulo State University

Abstract

Epidemiological studies have shown that between 40 to 60% of cows will suffer from one or more clinical episodes of disease within the first 60 days postpartum (Santos et al., 2010). Studies have shown that specific nutritional supplements such as essential fatty acids (Thatcher et al., 2011), organic selenium (Rutigliano et al., 1998; Cerri et al., 2009) and vitamin B complex molecules (Juchem et al., 2012; Kaur et al., 2016) have been positively associated with health and reproduction. This manuscript will show that there are evidence that lactation and inflammation negatively alters the gene expression profile of the uterus-conceptus communication. In addition, some nutritional supplements during the transition period could play a direct or indirect role in this communication resulting in improved fertility. It is often less clear the exact mechanism by which some of these supplements work to improve conception rates, which could explain some variation in study results. However, transition supplements such as fatty acids, vitamin B complex (alone or in combination), and some microminerals have generally resulted in mild to moderate improvements in reproduction.

Résumé

Des études épidémiologiques ont montré qu'entre 40 % et 60 % des vaches souffriront d'au moins un épisode clinique de maladie dans les 60 premiers jours postpartum (Santos et al., 2010). Des études ont montré que certains compléments nutritionnels, tels qu'acides gras essentiels (Thatcher et al., 2011), sélénium organique (Rutigliano et al., 1998; Cerri et al., 2009) et molécules du complexe vitaminique B (Juchem et al., 2012; Kaur et al., 2016), ont été associés à une amélioration de la santé et de la reproduction. Dans le présent document, nous montrerons qu'il existe des preuves que la lactation et l'inflammation ont des répercussions négatives sur le profil

d'expression génique du dialogue utéro-embryonnaire. De plus, durant la période de transition, certains compléments nutritionnels pourraient, directement ou indirectement, jouer un rôle dans ce dialogue pour améliorer la fécondité. Souvent, on ne sait pas exactement comment certains de ces compléments parviennent à améliorer les taux de conception, et c'est ce qui pourrait expliquer certaines variations dans les résultats d'étude. Toutefois, les compléments utilisés en période de transition, comme les acides gras, les vitamines du complexe B (seules ou en combinaison) et certains microminéraux, ont généralement produit des améliorations légères à modérées de la performance reproductrice.

Inflammation-stress-disease and reproduction

Greater milk production has been associated with physiological changes that can reduce fertility such as accentuated negative energy balance (Wathes et al., 2007), lower plasma concentrations of progesterone and estradiol (Wiltbank et al., 2006), and disruption of the growth hormone-insulin-like growth factor 1 axis in the liver (Kobayashi et al., 1999). Unfortunately, the mechanisms that lead to this embryonic and fetal loss are not understood at the molecular level. Sartori et al. (2002) observed a greater recovery of good quality embryos from heifers and dry cows than from lactating cows in single ovulating animals. During embryo transfer experiments it has been reported that pregnancy rates are lower when recipients are lactating cows compared to non-lactating cows or heifers (Hasler, 2001; Wilson et al., 2006). Combined, these studies suggest that lactation can reduce the quality of both embryos and endometrium.

Lactating dairy cows are subjected to a variety of challenges including metabolic and environmental stressors, particularly during the early postpartum period. Epidemiological studies have shown that between 40 to 60% of cows will suffer from one or more clinical episodes of disease within the first 60 days postpartum (Santos et al., 2010). This number of health disorders, that likely masks an even greater number of sub-clinical diseases, is thought to be associated with the energetic requirement for milk production and consequent metabolic stress. The drastic reduction in embryo development and conception rates and the increase in pregnancy losses (Santos et al., 2010) in cows that have experienced one or more health disorder episodes is a poorly understood area central to the causes for reproductive failure in dairy cows. The gene expression profile of early embryo development has been extensively described in the mouse (Zeng et al., 2004; Wang et al., 2013) but has only recently been studied in the bovine (Vallée et al., 2009). The plethora of genes and differentially expressed transcripts from the oocyte to the blastocyst stage emphasizes the complexity of signalling pathways necessary for successful embryo development. From fertilization to term, several molecules and systems apparently work in synchrony to prepare the uterus to receive and maintain the conceptus. Interferon-tau is synthesized by the trophectoderm cells of the conceptus and is essential for the maintenance of the corpus luteum during pregnancy (Hansen et al., 1985). The IFN-tau induces an array of changes in the uterus by promoting the expression of interferon stimulated genes (Spencer et al., 2008), which are related to cell remodelling, adhesion and invasion, cell orientation and polarization, angiogenesis, and transporters of glucose and lipids are mostly up-regulated by pregnancy and progesterone (Bauersachs et al., 2006; Forde et al., 2010). However, there is little information about the molecular changes caused by chronic stress and inflammation in the endometrium and the

conceptus. Providing this information is one of the major objectives of my research program. Recently, our group described changes in the global gene expression of the endometrium and the conceptus (Cerri et al., 2012) caused by lactation only. I was able to identify new genes related with embryonic development (*DKK1*, *RELN*, *PDK4*), and mainly with B and γδ T cells (*IGHG1*, *IGLL1*, *IGK*, and *TRD)* that have expanded our understanding of possible mechanisms related with embryonic losses.

Understanding the complex interaction of lactation, ovarian steroid milieu, health and stress/inflammatory status during embryo development is a key component to solve the problem of embryonic losses due to sub-optimal uterine environment. To further understand the intricate mechanisms and cross-communication between the endometrium and conceptus under challenged scenarios and the ability to improve the endocrine and uterine environment of the cow to increase the survival of the embryo are major goals of my research program. The dairy cow is a great model to study conditions related with metabolic stress and chronic health disorders normally observed in the early postpartum period. The extensive embryonic losses observed in dairy cows can greatly improve the knowledge of how the conceptus and the endometrium are modified to adapt to these adverse scenarios.

Transition from the nonlactating pregnant state to nonpregnant lactating state requires a dairy cow to drastically adjust her metabolism so that nutrients can be partitioned to support milk synthesis, a process referred to as homeorhesis (Bauman and Currie, 1980). A sharp increase in nutrient requirements generally occurs at the onset of lactation, when feed intake is usually depressed, which causes extensive mobilization of body tissues, particularly body fat, but also amino acids, minerals and vitamins (Santos et al., 2010). As consequence of the impaired immune competence during early lactation, the incidence of infectious diseases is substantial. A study evaluating health disorders in the first 2 months postpartum in 5,719 dairy cows in 5 confined dairies found that 44.2% of the cows had at least one clinical problem and 17.2% had at least two clinical problems (Santos et al., 2010). Incidence of individual diseases were: calving related problems, 14.6%; metritis, 16.1%; clinical endometritis, 20.8%; fever, 21.0%; mastitis, 12.2%; ketosis, 10.4%; lameness, 6.8%; digestive problems, 2.8%; and pneumonia, 2.0%. In another study evaluating health disorders before first breeding postpartum in 957 cows in 2 grazing dairies, the incidence of clinical diseases was 37.5%, the incidence of subclinical problems was 59.0%, and only 27.0% of the cows did not experienced any type of healthy problem. Incidence of individual diseases were: calving problem, 8.5%; metritis, 5.3%; clinical endometritis, 15.0%; subclinical endometritis, 13.4%; mastitis, 15.3%; respiratory problems, 2.5%; digestive problems, 4.0%; lameness, 3.2%; elevated NEFA 20.0%, subclinical ketosis, 35.4%; and subclinical hypocalcemia, 43.3%. In addition, of all the cows that leave a dairy farm because of culling or death, almost 12% do so in the first 3 weeks postpartum, and 24% in the first 2 months of lactation (Godden et al., 2003), with the majority being associated with health problems. Diseases have great significance for reproduction in dairy herds because of the high prevalence and because of the association with other fertility stressors such as anovulation and low BCS (Santos et al., 2009; Santos et al., 2010).

The biological mechanisms by which diseases postpartum impair reproduction, however, are not clearly understood. Most studies are of epidemiological nature and the overwhelming majority associates negative effects of diseases during early lactation with reduced P/AI or extended intervals to pregnancy. Important questions remain on how diseases affect developmental biology,

whether diseases that are of uterine or non-uterine origin affect fertility differently, the timing of disease occurrence relative to breeding, and the role of other associated risk factors that depress fertility such as anovulation and low BCS that are more prevalent in cows that suffer disease. Altogether, the biology that underlies subfertility attributed to diseases remains elusive and deserves investigation.

In confinement dairies, pregnancy per AI was reduced from 51.4% in healthy cows to 43.3% in cows with 1 clinical disease postpartum, and to 34.7% in cows with 2 or more cases of clinical diseases postpartum (Santos et al., 2010). Moreover, pregnancy loss between days 30 and 60 after AI increased from 8.9% in healthy cows to 13.9% in cows with 1 clinical disease postpartum, and to 15.8% in cows with 2 or more cases of clinical diseases postpartum. In grazing dairies, pregnancy per AI decreased from 66.9% in healthy cows to 56.5% in cows with 1 clinical disease postpartum, and to 40.8% in cows with 2 or more cases of clinical diseases postpartum (Ribeiro et al., 2013). Furthermore, pregnancy loss between days 30 and 65 after AI increased from 9.2% in healthy cows to 12.3% in cows with 1 clinical disease postpartum, and to 26.7% in cows with 2 or more cases of clinical diseases postpartum. While most of the diseases, approximately 75% (Ribeiro et al., 2016), are diagnosed in the first month postpartum, most of the first breeding postpartum occurs after the second month in lactation. Therefore, in some way diseases have carryover effects on fertility of dairy cows but the biological mechanisms involved are not completely understood.

In general, cattle affected by diseases have reduced appetite, increased body weight loss, and altered partitioned of nutrients (Gifford et al., 2012). The reduced appetite after establishment of disease seems to be a conserved behavior in mammals and birds that is explained by the communication between immune system and brain (Dantzer and Kelley, 2007; McCusker and Kelley, 2013). Proinflammatory cytokines produced in the infected tissue act in the brain to induce common symptoms of sickness, a phenomenon that in behavioral science is called cytokine-induced sickness behavior (Dantzer and Kelley, 2007). The action of cytokines could occur through neuronal communication or through circulation and leakage of blood brain barrier (McCusker and Kelley, 2013). The physiologic response of fever and the behavior responses such as reduced appetite, sleepiness, and unsocial behavior are believed to facilitate recovery and improve survival. Nonetheless, in postpartum dairy cows, those events would worsen the energy balance with additional consequences for the immune system.

Infection not only reduces energy intake but also increases energy expenditure, considering that mounting an immune response against infection has an energetic cost (Hotamisligil and Erbay, 2008). This energy cost takes away resources from other physiological processes including production, reproduction and perhaps immune system itself (Gifford et al., 2012). In a study estimating the energy expenditure for immune defense against pneumonia, fever-induced increase in metabolic rate accounted for up 90% of the energy costs (Romanyukha et al., 2006). This would be in agreement with the observation that puerperal metritis, when cows not only present massive inflammation of the uterus, but also systemic signs of disease such as anorexia and fever, have a greater impact on animal physiology than metritis without fever (Lima et al., 2014).

Reduced energy intake, increased energy expenditure for building an immune response, and altered nutrient partition resulted from inflammation can potentially worsen the energy balance

and immune cell function in transition cows, which in turn further increases susceptibility to metabolic and infectious diseases, creating a vicious cycle that includes reduction in energy balance, impaired immune cell competence, increased incidence of diseases, and back to reduction in energy balance. Not surprisingly, occurrence of one health problem increases the susceptibility to other problems, and has serious consequences for productive and reproductive performance (Santos et al., 2010; Ribeiro et al., 2013).

Studies: Stressors and reproductive tissues

The objective of this section is to describe a series of studies performed by our group and close collaborators. The rationale is to show the reader how recent experiments (both epidemiological and treatment induced changes) varying in the degree of stress imposed on the animal proves the significant effect of inflammation/disease/stress on reproductive tissues, particularly the endometrium and the early embryo. These research is focused on identifying and characterizing the basic mechanisms responsible for decreased reproductive efficiency in lactating dairy cows. To begin, a study comparing lactating vs. non-lactating animals (Cerri et al., 2012; Thompson et al., 2012) was performed in order to check how lactation itself (minimizing the metabolic effect) induce changes in reproductive tissues. Most recently, a study the effects of elevated concentrations of ammonia and urea on embryo development *in vitro* (Gunaretnam et al., unpublished) and on gene expression in the endometrium *in vivo.* A second part will describe two experiments in which LPS, a potent inducer of an inflammatory state, was consecutively administered to heifers and cows after AI. The results will show how this induced pro-inflammatory state affects d 15 embryos and the gene expression of the endometrium at that stage. Lastly, a compilation of several studies (Ribeiro et al., 2016) unveiled a unique dataset that clearly illustrates the significant carryover effects of disease episodes in the first month post-partum on later embryonic development and uterine environment.

Studies 1. Lactation

Lactation and Pregnancy 1 (Cerri et al., 2012): Objectives were to determine effects of lactation and pregnancy on endometrial gene expression on day 17 of the estrous cycle and pregnancy. Heifers (n=33) were assigned randomly after parturition to lactating (L, n=17) or non-lactating (NL, n=16) groups. Cows were subjected to an ovulation synchronization program for a timed artificial insemination (TAI); 10 cows in L and 12 in NL were inseminated. Slaughter occurred 17 days after the day equivalent to TAI, and intercaruncular endometrial tissues collected. Gene expression was determined by DNA microarray analysis for pregnant (L, n=8; NL, n=6) and non-inseminated cyclic (L, n=7; NL, n=4) cows. Differentially expressed genes were selected with *P*-value < 0.01 and absolute expression > 40. In addition, a fold effect > 1.5 was used as a criterion for genes affected by pregnancy. There were 210 genes differentially regulated by lactation (136 down-regulated and 74 up-regulated) and 702 genes differentially regulated by pregnancy (407 down-regulated and 295 up-regulated). The interaction effect of pregnancy and lactation affected 61 genes. Genes up- and down-regulated in pregnant cows were associated with several gene ontology (GO) terms, such as defense response and interferon regulatory factor, cell adhesion and extracellular matrix. The GO analyses of up- and down-regulated genes of lactating cows revealed terms related to immunoglobulin-like fold, immune response, COMM domain and non-membrane

bounded organelle. A number of upregulated genes by lactation such as *IGHG1, IGLL1, IGK*, and *TRD* were all related to immune function, particularly for B cells and γδ T cells. Developmental genes related to limb and neural development and glucose homeostasis (*DKK1, RELN, PDK4*) were down-regulated by lactation**,** whereas an interaction was also detected for *RELN*. The stated genes associated with immune function and developmental genes expressed in the endometrium impacted by lactational state are possible candidate genes for interventions to improve fertility of lactating dairy cows.

Summary: In conclusion, the effect of lactation on the endometrium unveiled possible new candidate genes responsible for the sub-fertility in lactating dairy cows. *FCGRT, IGHG1, IGLL and TRD* were up-regulated by lactation, but not altered by pregnancy. These genes indicate a possible increase in B-lymphocyte and γδ T cell activity that could negatively impact the fine immunological balance of the uterus necessary to accept the semi-allograph embryo. In addition, lactation affected *DKK1* and *RELN*, two genes involved with embryonic neural and limb development, by countering the effects of pregnancy on these genes. The Wnt signalling pathway was down regulated by pregnancy, and the increase in *DKK1* by lactation may prove an important process that regulates embryonic loss in lactating dairy cows. There was a plethora of coordinated expression of genes necessary to support pregnancy. The regulation of different immune functions seems to be the most important event in the d 17 pregnant endometrium as observed by the several genes and GO terms related to the immune system, and lactation is likely to affect some of these mechanisms. Cell adhesion, represented by cadherins, claudins, collagens and L-galectins, was a function mostly down regulated by pregnancy at this stage of pregnancy and may indicate a strong tissue remodelling as well as a window of time that facilitates the flow of substrates to the uterine lumen to support the elongating embryo. The implication of lactation on the Wnt signalling pathway could also interfere with the endometrium remodelling, as well as, along with *RELN*, impact embryonic differentiation and development. As a consequence of the great activity in endometrial tissue on d 17 of gestation, genes related to carbohydrate, lipid and amino acid metabolism and transport are all up-regulated by pregnancy, but the up-regulation of *PDK4* by lactation is indicative of a negative role on glucose homeostasis caused by lactation (Figure 1).

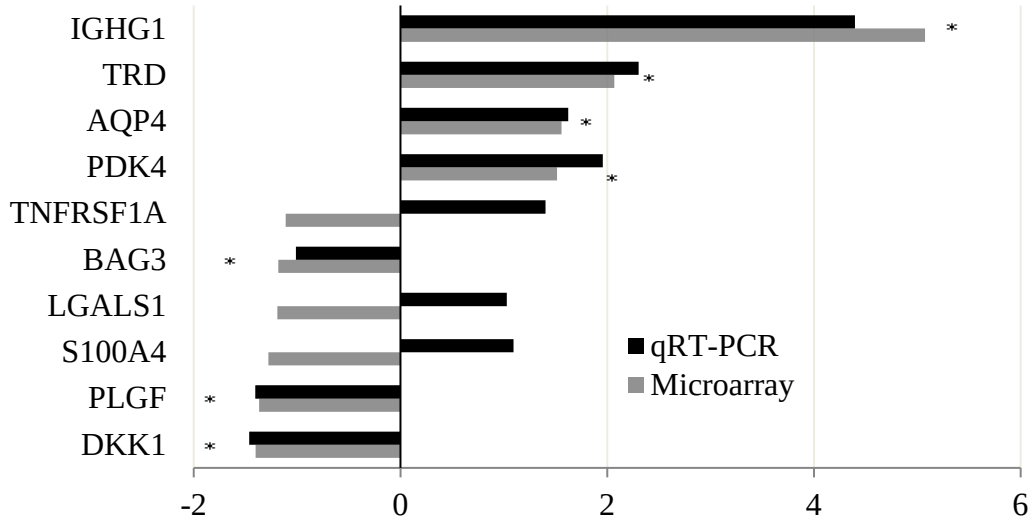


Figure 1. Validation of the microarray results using qRT-PCR of the expression of selected transcripts that were initially found up- or down-regulated in response to effects of lactation (lactating vs. non-lactating). Beside each bar,*denotes that *P* values from the microarray and qRT-PCR analyses had agreed.

Lactation and Pregnancy 2 (Thompson et al., 2012): Objectives were to characterize postpartum metabolic and hormonal differences between non-lactating and lactating dairy cows, evaluate lactation and pregnancy effects on endometrium and conceptus gene expression, and characterize associations between conceptus and endometrial expression of genes in early pregnancy (d 17). Pregnant heifers were assigned randomly after calving to a lactating group (L, n=17) and a nonlactating group (NL, n=16). The L cows were fed a TMR (1.65 Mcal NEL/kg, 16.5% CP) ad libitum. NL cows were fed a maintenance ration (1.45 Mcal NEL/kg, 12.2% CP) once per day. All cows were pre-synchronized and enrolled in a timed artificial insemination (TAI) protocol; 10 cows in the L and 12 in the NL were TAI. On d 17 after GnRH/TAI, cows were slaughtered and endometrial and conceptus tissues collected. The Affymetrix Bovine Genome DNA Microarray was used to assess conceptus and endometrial gene expression. Temporal changes in BCS and BW did not differ between L and NL cows. L cows had higher body temperature than NL cows (38.4 vs 38.2ºC), and NL cows cycled earlier than L (26.3 vs 34.7 DIM). Plasma concentrations of NEFA and insulin did not differ between NL and L. However, cows in L group had greater plasma concentrations of BHBA (4.90 vs 2.97 mg/dL), BUN (11.6 vs 6.5 mg/dL), and lower concentrations of glucose (74.0 vs 79.9 mg/dL) than NL cows. The IGF-1 was lower for L compared with NL (140.5 vs 198.2 ng/mL) and was greater for pregnant compared to cyclic (191.0 vs 147.6 ng/mL) cows. Concentration of progesterone from GnRH or TAI (d 0) until d 17 was lower for L cows than NL cows. Gene expression analyses indicated that all conceptuses (n = 13) expressed Pregnancy Associated Glycoprotein (PAG) genes P*AG2*, *PAG8*, *PAG11* and *PAG12*. The same PAG family genes were observed in the endometrium of some pregnant cows. Simple and standard partial correlation analyses detected associations of conceptus *PAG11* with prostaglandin regulatory genes. Moreover, in the endometrium, *PAG11* was associated with prostaglandin regulatory genes, trophoblast cell specific genes, genes involved in invasion and

implantation, and progesterone regulatory genes. In conclusion, lactation altered metabolic status, delayed initiation of cyclicity and lowered concentrations of progesterone in pregnant cows. Early expression of PAG genes in the conceptus and endometrium of pregnant cows may contribute to successful development of early pregnancy.

Summary: In conclusion, lactation altered metabolic status even though BW and BCS were the same between L and NL. Lactation delayed initiation of cyclicity and lowered concentrations of progesterone in pregnant L cows during a programmed period following an induced ovulation. Early expression of PAG genes within the conceptus and endometrium of pregnant cows and its association with other genes infer a possible role of PAG in pregnancy maintenance and implantation by regulation of embryo development, trophoblast cell invasion, immune regulation, and prostaglandin metabolism.

Studies 2. LPS Challenges

LPS 1. Intravenous (Fernandes et al., 2015). This study aimed to evaluate the effect of repeated intravenous lipopolysaccharide (LPS) stimulus in endometrial gene expression of candidate transcripts of non-lactating heifers on late luteal phase. Heifers (n=22; 11 mo of age) were synchronized by the ovsynch protocol and enrolled into control group (CON; n=11) that received sterile saline solution i.v., or LPS group (LPS; n=11) submitted to repeated LPS injections i.v. (0,1; 0,25; 0,5; 0,75; 1,0; 1,25 ug/Kg) starting two d after AI (d0), then every other d. At each LPS injection, rectal temperatures were measured during six h. Blood samples were collected from the d-1 to d13, for analyses of TNF-α, haptoglobin and progesterone, and WBC count and differential. On d15, endometrium tissue biopsies were taken and kept at -80°C until qRT-PCR analysis of 30 target genes related to immune system, adhesion molecules and endometrium receptivity. Data was checked for normality and analysed by ANOVA for repeated measures using proc MIXED and UNIVARIATE. After each LPS injection, temperature was greater in the first 6h in the LPS compared with CON group ($P<0.05$). Both TNF-α ($P=0.05$) and haptoglobin ($P<0.01$) were increased in the LPS group with significant ($P<0.05$) treatment by d interactions. Total leukocyte count was not different between treatments ($P=0.29$), but differential count was increased for neutrophils, band cells and monocytes, but decreased for lymphocytes and eosinophil in LPS compared with CON group ($P<0.01$). Progesterone concentrations during the experimental period were not different between treatments. Out of the 30 target genes analyzed, only three transcripts were differentially expressed. IDO ($P=0.04$; Fold=0.48) and PTX3 ($P=0.01$; Fold=0.38) were down-regulated, whereas MX1 ($P=0.02$; Fold=2.85) was up-regulated in the LPS group. Concentration of IFN-tau in the uterine lavage was similar between treatments. Sequential LPS injections was able to induce a systemic pro-inflammatory state with limited, but strong effect in gene expression of transcripts related to the immune system, suggesting a possible explanation for sub-fertility related with health disorders in dairy cows.

LPS 2: Intrammary (Campos et al.). In addition of consequences of clinical mastitis on productive parameters, this disease has a negative impact on reproductive performance of dairy cows due to inflammatory and immune responses related with the mammary gland infection that compromises fertility, pregnancy establishment and maintenance. Some molecules produced by the pathogens or immune reactions by the host , such as cytokines, acute phase proteins (APP) and other active

biomolecules, may influence oocyte maturation, fertilization and early embryo development. Stress factors, such as clinical mastitis, affects reproductive efficiency by altering estrous behavior and ovarian follicular growth in dairy cows, as well as the influence on precise timings of reproductive hormone release within the follicular phase and the absence or delay on luteinizing hormone (LH) surge onset. The aim of the study was to evaluate the effect of inflammatory response in consequence of CM, induced by lipopolysaccharide (LPS) endotoxin of *Escherichia coli* infusion in the mammary gland, on endometrial gene expression of candidate transcripts of lactating Holstein cows during pre-implantation phase of early embryonic development. In this study, LPS was infused in the mammary gland on days 5 and 10 post-AI. All the physiological and systemic inflammatory markers were modified in the LPS group. The parameters of reticular temperature, haptoglobin and TNF-α in plasma, and SCC in milk were all elevated in the post-infusion period ($P<0.01$). Milk production was decreased by LPS infusion, but concentration of progesterone in plasma was similar between groups. Although, the gene expression portion of this study is still incomplete, the size and weight of the recovered conceptuses were modified due to intramammary LPS infusions.

Summary: The expression about three times less of IDO and PTX3 genes observed in heifers of LPS group compared to the CON, may signal a possible action of pro-inflammatory mediators involved in the systemic inflammatory response over the anti-inflammatory profile required to establish immune tolerance to the embryo . The results corroborate with the study of Walker et al (2012) that evaluated the endometrial gene expression during early pregnancy in cows considered fertile and sub-fertile, and observed that in the sub-fertile group, IDO and PTX3 genes were also expressed in smaller amount than in the fertile cows. Besides the suppression of genes responsible for anti-inflammatory endometrial profile during the early pregnancy, such as IDO and PTX3, the apparent deregulation in the MX1 expression, expressed approximately 3.5 times more in LPS group than the CON, supporting the idea of a possible change in endometrial immunological profile, suggeting an pro-inflammatory profile. In spite of these findings, the lack of major changes in IFN-tau concentrations in the uterine flush (intravenous) and in conceptus size (intrammamary) and the important, but relatively small changes in the endometrium gene expression demonstrates the large gap in knowledge relating health and inflammation with fertility. It is clear, in the case of mastitis as observed in the next study showed, of the remarkable consequences of sub-optimal health in fertility. However, at this point, there is no clear rationale able to explain from the early conceptus and endometrium environment angle, the molecular and cellular causes for such effects.

Somatic cell count and mammary gland infection on fertility (Barbosa et al.): The objectives of these studies were to determine the sensitivity (**Se**) and specificity (**Sp**) of different somatic cell count (**SCC**) thresholds to identify subclinical mastitis and to assess the impact of mastitis-causing bacteria and SCC during timed embryo transfer (**TET**) protocol on pregnancy per embryo transfer (**P/ET**) in recipient lactating dairy cows. In study 1, 2,516 Holstein and Girolando lactating cows from 8 herds were enrolled. Two milk samples at randon stage of lactation were collected from each cow to evaluate SCC and bacteriological culture. In study 2, 1,397 Girolando lactating cows were subjected to a TET protocol. Milk samples were collected two days before the TET for bacteriological culture and SCC. Pregnancy diagnoses were performed on days 31 and 66 after TET. The animals were grouped according to the NMC recommendations: Gram-positive environmental (**EV+**; *Bacillus spp., Enterococcus spp., Streptococcus spp, Micrococcus spp.*), Gram-negative environmental (**EV-**, *Citrobacter freundii*, Coliforms, *Enterobacter spp.*,

Klebsiella spp., Proteus spp., Pseudomonas spp.), Gram-positive contagious (**C+**; *Corynebacterium bovis, Staphylococcus aureus, Streptococcus agalactiae*), coagulase-negative staphylococci (**CNS**) and control (no bacteria growth). In study 1, the overall prevalence of IMI was 61.8% and the sensitivity and Sp. of selected SCC thresholds varied according to the group of pathogen. The greater Se. (78.1%) was observed with the 100,000 cells/mL threshold. In study 2 bacterial growth reduced (P <0.01) P/ET at 31 and 66 d. P/ET was lower (P<0.05) at 31 d in [EV-] (30.1% [68/201]) and [EV +] (29.9% [24/82]) groups and tended (P = 0.09) to be lower in the group [C +] (36.6% [85/245]) than the control (44.0 [367/869]). Cows with SCC greater than 400,000 cells/mL had lower (P<0.01) P/ET (30.4% [75/247]) than animals with SCC lower than 200,000 cells/mL (40.8% [292/716]). The use of a single SCC analysis may not be an effective parameter to detect IMI, because Se. and Sp. were low. Elevated SCC and intramamary bacterial isolation reduced the P/ET of recipients lactating dairy cows.

Summary: The use of a single SCC analysis per animal may not be the most effective parameter to detect IMI since the Se. and Sp. Were low in the thresholds studied. High SCC and intramammary bacterial isolation reduce the P/ET in lactating dairy cows, suggesting that some of the effects are on the uterus or on the embryo after d7.

Studies 3. Disease

Disease 1: Embryonic Development to the Morula Stage (Ribeiro et al., 2016). A total of 597 cows out of 635 (94%) had synchronized ovulation following AI and had the uterus flushed on d 5 or 6 after AI for recovery of ova or embryos. From those, 419 flushings (70.2%) resulted in a recovered ovum-embryo of which 347 (82.8%) were cleaved. A total of 280 embryos were considered to be live, whereas 67 were degenerated. Live embryos represented 66.8% of all ova-embryos recovered and 80.7% of all embryos recovered. A total of 233 ova-embryos were classified as high quality, which represented 55.6% of all ova-embryos and 67.1% of embryos recovered.

The incidence of disease before AI was 41.9%. The incidences of uterine disease (UTD) and non-uterine disease (NUTD) were 22.0 and 28.8%, respectively. Incidence of disease before AI reduced the proportion of cleaved, live, and high quality embryos relative to ova-embryos recovered (Figure 2), as well as the proportion of live and high quality embryos relative to embryos recovered. The results were similar when data were modeled according to UTD and NUTD separately, except for the proportion of cleaved ova, which were affected by UTD but not by NUTD.

Disease 2: Preimplantation Conceptus Development and Transcriptome (Ribeiro et al., 2016). Interferon-τ was detected in the uterine fluid in 70 of 148 cows (47.3%), and these cows were considered pregnant. From those, 52 conceptuses were recovered. The incidence of disease before AI was 45.3%. The incidences of UTD and NUTD were 32.4 and 21.6%, respectively. Disease did not affect (P = 0.93) pregnancy on d 15-16, but resulted in shorter conceptuses and reduced concentration of IFN-τ in the uterine flush (Figure 3). Similar results were observed when data were modeled according to UTD or NUTD. Cows diagnosed with UTD had shorter (P < 0.01) conceptus than those without uterine disease. Cows with NUTD also had shorter (P = 0.02)

conceptus than those without NUTD. Because of the shorter conceptus, cows with UTD ($P = 0.06$) and those with NUTD ($P = 0.07$) tended to have reduced concentrations of IFN-τ in the uterine fluid. Disease reduced ($P < 0.05$) the concentration of progesterone in all cows (Figure 3). Nonetheless, progesterone in the first 15 d of gestation in pregnant cows did not differ with disease (Figure 3). Transcriptome analyses revealed few genes differently expressed between conceptuses recovered from cows previously diagnosed and those not diagnosed by NUTD before AI. Only 7 transcripts were differently expressed, 4 downregulated and 3 upregulated in d 15 conceptuses from NUTD cows. On d 16 conceptuses, 35 transcripts were differently expressed, 9 downregulated and 26 upregulated in NUTD cows. Functional analysis revealed that the main diseases and disorders associated with the differently expressed genes on d 16 conceptuses were inflammatory disease (11 molecules; *OP* < 0.04), immunological disease (11 molecules; *OP* < 0.05), and connective tissue disorders (8 molecules; *OP* < 0.05). Three potential upstream regulators of the changes in transcriptome were identified and they all had predicted increased activity. These were lipopolysaccharide (*OP* = 8.5E-04, *ZS* = 2.4), interferon-γ (*OP* = 4.1E-03, *ZS* = 2.4), and tumor necrosis factor (*OP* = 5.7E-02, *ZS* = 2.2). Two potential downstream effects of the differently expressed genes with very small *OP* values were activation of cells (*OP* = 4.2E-05, *ZS* = 1.7) and systemic autoimmune syndrome (*OP* = 4.8E-05, effect not predicted). In addition, the most significant canonical pathways included OX40 signaling pathway (*OP* = 5.0E-05), type I diabetes mellitus signaling (*OP* = 6.5E-04), antigen presentation pathway (*OP* = 1.1E-03), graft-versus-host disease signaling (*OP* = 1.1E-03) and allograft rejection signaling (*OP* = 1.4E-03).

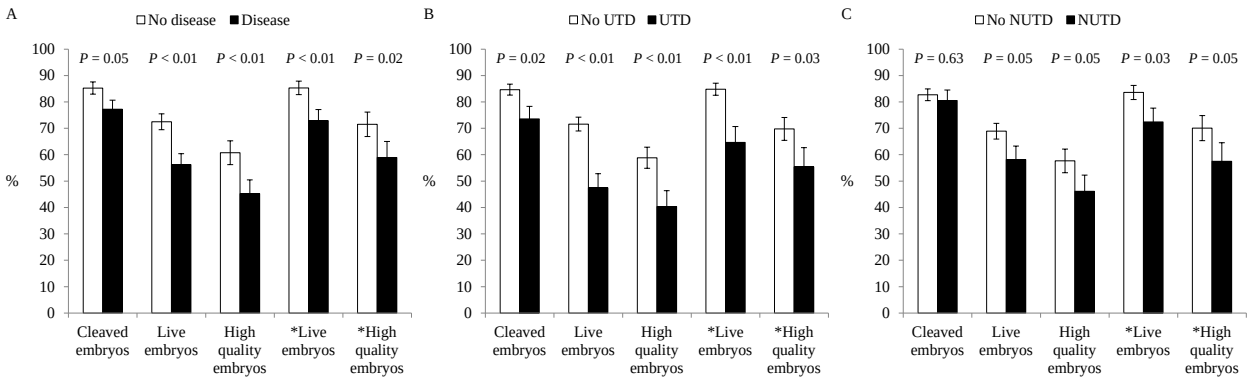


Figure 2. Cleaved embryos, live embryos (grades 1 to 3), and high quality embryos (grades 1 and 2) as percentage of ova-embryos recovered (no asterisk mark) or as percentage of embryos recovered (asterisk mark), according to the incidence of disease (Panel A), uterine diseases (**UTD**; Panel B), and non-uterine diseases (**NUTD**; Panel C). Error bars represent the SEM.

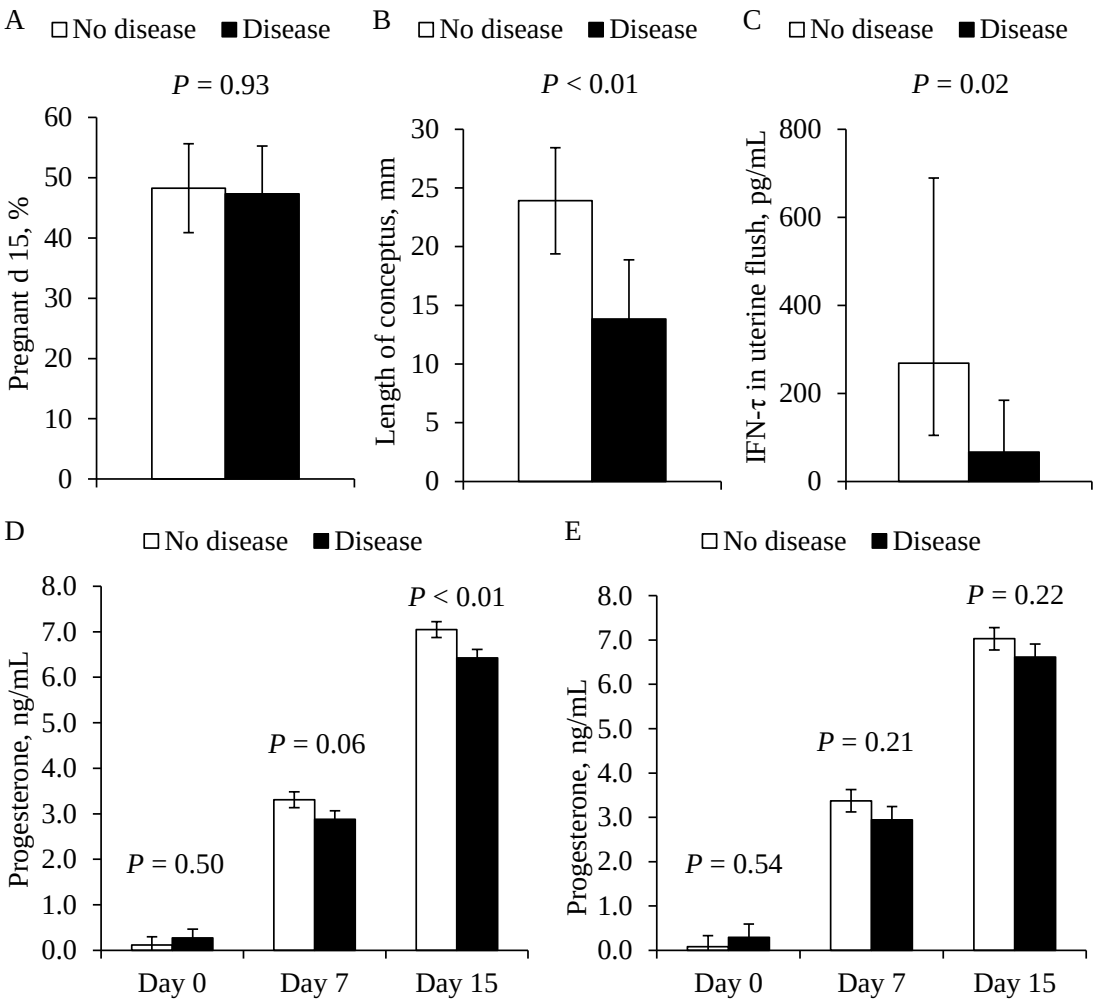


Figure 3. Pregnancy per AI on d 15 based on detection of interferon-τ (IFN-τ) in the uterine flush (Panel A); length of the recovered intact conceptuses (Panel B); concentration of IFN-τ in the uterine flush of pregnant cows (Panel C); progesterone concentration on d 0, 7 and 15 relative to AI in all cows (Panel D) or in pregnant cows (Panel E) according to the incidence of disease before AI. Error bars represent the SEM for all panels except for panel C, which represent the 95%

Studies 4. Vitamin B

Effect of rumen protected vitamin B complex on metabolic parameters, milk production and d 14 conceptus and endometrial outcomes. By Kaur et al., 2016. This last study sample is an example of one study recently performed by our group showing some possible mechanism of action on how vitamin B can improve the communication between the conceptus and the endometrium. A previous study have shown (Juchem et al., 2012).

The aim of this project was to determine the effects of a rumen-protected vitamin B complex supplementation (VIT B) compared with a control diet containing no supplementation (CON) on:

milk production and components, concentrations of BHBA, haptoglobin and progesterone in plasma, ovarian dynamics and day 14 conceptus and endometrial outcomes. Fifty-one multiparous Holstein cows from the herd at the UBC Dairy Education and Research Centre were enrolled into the study 3 weeks prior to parturition and were randomly assigned to one of the two treatments. Biweekly blood samples, weekly milk samples and daily feed intake were collected. Cows were enrolled onto a double-ovsynch protocol at 33±3 days post-partum and inseminated by timed artificial insemination (AI). Ovarian structures were monitored and measured using *per rectum* ultra-sonography. The uterus was flushed on day 14 post AI for conceptus collection and endometrial samples were collected at the same time. Data was analyzed by ANOVA using the GLM procedure of SAS. Overall, 42 cows were flushed and 13 embryos were collected. Vitamin B supplementation had no affect on the size of the embryo, ovulatory follicle size or CL size at embryo collection. Milk production and milk fat values were also similar between the two groups. BHBA and haptoglobin levels between the two groups were also identical. Analysis of expression of genes related to embryo development, immune system, adhesion and regulation of Vitamin B molecules showed that *OXTR*, *MUC5B*, *MUC1*, *IL1β*, *SPP*, *TRD*, *FZD8* and *FOLR1* genes were significantly upregulated in the VIT B group. *SELL*, *PLAU* and *MYH9* genes showed a tendency to be more upregulated in the endometrium of VIT B group compared to CON group (Figure 4).

Summary: In conclusion, strategic dietary vitamin B supplementation during the transition and early lactation did not affect major outcomes of production and reproduction in lactating dairy cows. However, benefits of vitamin B in fertility might potentially be linked to endometrial and conceptus gene expression. Genes associated with immune system, adhesion, nutrient transporters, Wnt signalling and embryo morphogenesis were different in cows receiving supplementation. The results suggest that Vitamin B supplementation has a downstream effect on the uterus by influencing molecular changes and hence can potentially improve pregnancy risk in multiparous animals.

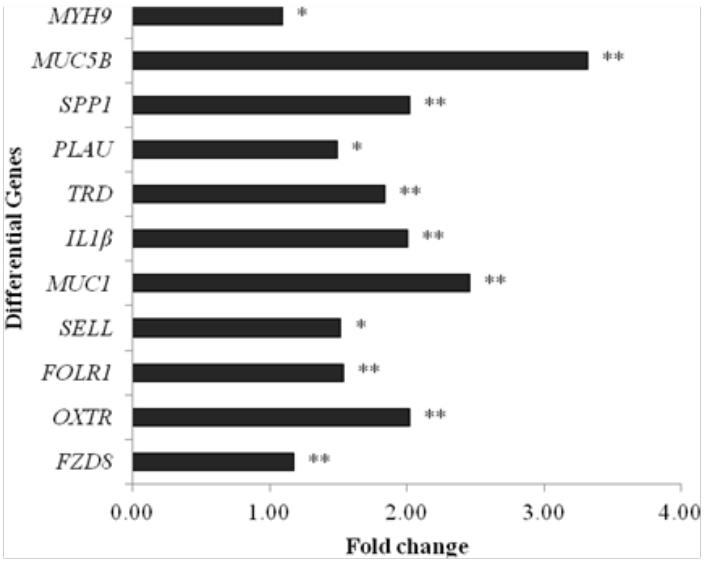


Figure 4. Relative fold change of differential genes between pregnant Vitamin B and Control animals (**P ≤ 0.05, *P = 0.1)

References

Bauersachs, S., S.E. Ulbrich, K. Gross, S.E. Schmidt, H.H. Meyer, H. Wenigerkind, M. Vermehren, F. Sinowatz, H. Blum, and E. Wolf. 2006. Embryo induced transcriptome changes in bovine endometrium reveal species-specific and common molecular markers of uterine receptivity. Reproduction 132:319-331.

Bauman, D. E. & W. B. Currie. 1980. Partitioning of nutrients during pregnancy and lactation: a review of mechanisms involving homeostasis and homeorhesis. J. Dairy Sci. 63:1514-1529.

Cerri, R.L.A.; Thompson, I.M.; Kim, I.H.; Ealy, A.D.; Hansen, P.J.; Staples, C.R.; Li, J.L.; Santos, J.E.P.; Thatcher, W.W. 2012. Effects of lactation and pregnancy on gene expression of endometrium of Holstein cows at day 17 of the estrous cycle or pregnancy. J. Dairy Sci. 95:5657-5675.

Dantzer, R., and K. W. Kelley. 2007. Twenty years of research on cytokine-induced sickness behavior. Brain Behav. Immun. 21:153-160.

Forde N., T.E. Spencer, F.W. Bazer, G. Song, J.F. Roche, and P. Lonergan. 2010. Effect of pregnancy and progesterone concentration on expression of genes encoding for transporters or secreted proteins in the bovine endometrium. Physiol. Genom. 41:53-62.

Gifford C.A., B. P. Holland, R. L. Mills, C. L. Maxwell, J. K. Farney, S. J. Terrill, D. L. Step, C. J. Richards, L. O. Burciaga-Robles, and C. R. Krehbiel. 2012. Growth and development symposium: Impacts of inflammation on cattle growth and carcass merit. J. Anim. Sci. 90:1438-1451.

Glaser, R. & J. K. Kiecolt-Glaser. 2005. Stress-induced immune dysfunction: implications for health. Nature Reviews Immunology. 5:243-251.

Godden, S., P. Rapnicki, S. Stewart, J. Fetrow, A. Johnson, R. Bey, R. Farnsworth. 2003. Effectiveness of an internal teat seal in the prevention of new intramammary infections during the dry and early-lactation periods in dairy cows when used with a dry cow intramammary antibiotic. J. Dairy Sci. 86:3899-3911.

Hansen, P.J., R.V. Anthony, F.W. Bazer, G.A. Baumbach, and R.M. Roberts. 1985. In vitro synthesis and secretion of ovine trophoblast protein-1 during the period of maternal recognition of pregnancy. Endocrinology 117:1424-1430.

Hasler, P.F. 2001. Factors affecting frozen and fresh embryo transfer pregnancy rates in cattle. Theriogenology. 56:1401-1415.

Hotamisligil GS, Erbay E. 2008. Nutrient sensing and inflammation in metabolic diseases. Nat Rev Immunol. 8(12):923–34.

Juchem, S.O., Robinson, P.H., and Evans, E. 2012. A fat based rumen protection technology post-ruminally delivers a B vitamin complex to impact performance of multiparous Holstein cows. Anim. Feed Sci. Tech. 174:68-78.

Kaur, M., Hartling, I., Burnett, T.A., Polsky, L., Donnan, C., Leclerc, H., Cerri, R.L.A. 2016. Effect of rumen protected vitamin B complex on metabolic parameters, milk production and d 14 conceptus and endometrial outcomes. J. Dairy Sci (

Kobayashi, Y., C. K. Boyd, C. J. Bracken, W. R. Lamberson, D. H. Keisler, and M. C. Lucy. 1999. Reduced growth hormone receptor (GHR) messenger ribonucleic acid in liver of periparturient cattle is caused by a specific down-regulation of GHR 1A that is associated with decreased insulin-like growth factor I. Endocrinology 140:3947-3954.

Lima, F. S., A. Vieira-Neto, G. S. F. M. Vasconcellos, R. D. Mingoti, E. Karakaya, E. Solé, R. S. Bisinotto, N. Martinez, C. A. Risco, K. N. Galvão, and J. E. P. Santos. 2014. Efficacy of ampicillin trihydrate

or ceftiofur hydrochloride for treatment of metritis and subsequent fertility in dairy cows. J. Dairy Sci. 97:5401-5414.

McCusker, R. H., and K. W. Kelley. 2013. Immune-neural connections: how the immune system's response to infectious agents influences behavior. J. Exp. Biol. 216:84-98.

Ribeiro E. S., F. S. Lima, L. F. Greco, R. S. Bisinotto, A. P. A. Monteiro, M. Favoreto, H. Ayres, R. S. Marsola, N. Martinez, W. W. Thatcher, and J. E. P. Santos. 2013. Prevalence of periparturient diseases and effects on fertility of seasonally calving grazing dairy cows supplemented with concentrates. J. Dairy Sci. 96:5682-5697.

Ribeiro, E.S., G. Gomes, L.F. Greco, R.L.A. Cerri, A. Vieira-Neto, P.L. Monteiro Jr., F.S. Lima, R.S. Bisinotto, W.W. Thatcher, and J.E.P. Santos. 2016. Carryover effect of postpartum inflammatory diseases on developmental biology and fertility in lactating dairy cows. J Dairy Sci. 99:2201-2220.

Romanyukhaa, A. A., S. G. Rudneva, and I. A. Sidorov. 2006. Energy cost of infection burden: An approach to understanding the dynamics of host-pathogen interactions. J. Theor. Biol. 241:1-13.

Santos, J.E.P., H.M. Rutigliano, and M.F. Sá Filho. 2009. Risk factors for resumption of postpartum cyclicity and embryonic survival in lactating dairy cows. Anim. Reprod. Sci. 110:207-221.

Santos, J.E.P., R.S. Bisinotto, E.S. Ribeiro, F.S. Lima, L.F. Greco, C.R. Staples, and W.W. Thatcher. 2010. Applying nutrition and physiology to improve reproduction in dairy cattle. Soc. Reprod. Fertil. Suppl. 67:387-403.

Sartori, R., G.J. Rosa, and M.C. Wiltbank. 2002. Fertilization and early embryonic development in heifers and lactating cows in summer and lactating and dry cows in winter. J. Dairy Sci. 85:2813-2822.

Spencer, T.E., O. Sandra, and E. Wolf. 2008. Genes involved in conceptus-endometrial interactions in ruminants:insights from reductionism and thoughts on holistic approaches.Reproduction 135:165-179.

Thompson, I. M., R. L. A. Cerri, I. H. Kim, A. D. Ealy, P. J. Hansen, C. R. Staples, and W. W. Thatcher. 2012. Effects of lactation and pregnancy on metabolic and hormonal responses and conceptus and endoametrial gene expression of Holstein dairy cattle. J. Dairy Sci. submitted.

Vallée, M., I. Dufort, S. Desrosiers, A. Labbe, C. Gravel, I. Gilbert, et al. 2009. Revealing the bovine embryo transcript profiles during early in vivo embryonic development. Reproduction. 138:95–105

Walker, C. G.; M. D. Littlejohn, M. D. Mitchell, J. R. Roche, S. 2012. Meier. Endometrial gene expression during early pregnancy differs between fertile and subfertile dairy cow strains. Physiological genomics, 44:47–58.

Wang, D., M. Garcia, R.S. Bisinotto, N. Martinez, F. S. Lima, L. F. Greco, J. H. Shin, A. M. M. Calaça, A. L. Ranieri, B. L. Artiaga, E. K. Ganda, G. C. Gomes, L. F. V. Becker, S. C. Soares, V. S. Rezende, M. A. Engstrom, J. E. P. Santos, and C. R. Staples. 2013. Effect of supplementing vitamin E and β-carotene to prepartum Holstein cattle on health and reproductive responses. J. Dairy Sci. 96 (Suppl. 1):253 (Abstr.).

Wathes, D. C., M. Fenwick, Z. Cheng, N. Bourne, S. Llewellyn, D. G. Morris, D. Kenny, J. Murphy, and R. Fitzpatrick. 2007. Influence of negative energy balance on cyclicity and fertility in the hight producing dairy cows. Theriogenology 68:232-241.

Wilson, R. D., P. M. Fricke, M. L. Leibfried-Rutledge, J. J. Rutledge, C. M. Penfield, and K. A. Weigel. 2006. In vitro production of bovine embryos using sex-sorted sperm. Theriogenology, 65:1007-1015.

Wiltbank, M., H. Lopez, R. Sartori, S. Sangsritavong, and A. Gumen. 2006. Changes in reproductive physiology of lactating dairy cows due to elevated steroid metabolism. Theriogenology 64: 17-29.

Zeng, F., Baldwin, D. A. & Schultz, R. M. 2004. Transcript profiling during preimplantation mouse development. Dev. Biol. 272:483–496.

Improving Health and Resistance of Animals in Swine and Dairy Production by Using an Innovative Nutritional Strategy Targeting Mitochondria

Améliorer la santé et la résistance des animaux en production porcine et laitière par l'utilisation d'une stratégie nutritionnelle novatrice ciblant les mitochondries

Jérôme Lapointe
Research Scientist
Sherbrooke R & D Centre
Agriculture and Agri-Food Canada
Sherbrooke, Qc, J1M 0C8
jerome.lapointe@agr.gc.ca

Abstract

To remain economically profitable in coming decades of high food demands, swine and dairy agriculture have to increase their production efficiency. Genetic selection for specific trait characters such as milk production, prolificacy or animal growth is already being implemented as a mechanism to achieve increased efficiency. However, genetic selection by itself was found unable to answer all expectations and was sometimes accompanied by undesired side effects such as decrease in longevity or susceptibility to diseases. Moreover, these consequences were amplified by the actual withdrawal of antibiotics as prophylactic treatments. This is especially evident when animals encounter metabolic stresses such as throughout the transition period for dairy cows, the gestation for gilts and the post-weaning period for piglets. During these periods, the energetic deficit coincides with dysfunctional immune responses, uncontrolled inflammation and oxidative stress conditions which are associated with infections and diseases. Mounting evidence indicates that mitochondria, the main cellular sources of energy, are closely related to oxidative stress, immune response, inflammation and bacterial pathogenesis. Interestingly, mitochondrial functions are quite sensitive to the quantity and variety of nutrients provided in diet. This recent recognition that nutrients have the ability to modulate specific mechanisms underlying physiological functions has prompted a revolution in the field of nutrition and favored the undertaking of numerous nutrigenomics studies. Nutrigenomics approaches targeting mitochondria appear promising to show that not only are certain nutrients essential, but also that specific quantities of particular nutrients are necessary to optimize health, robustness and longevity of animals in swine and dairy production.

Résumé

Pour demeurer rentables dans le contexte forte demande alimentaire des prochaines décennies, les productions porcine et laitère doivent améliorer l'efficacité de leurs activités. La sélection génétique en faveur de certains caractères, tels que la production de lait, la prolificité ou la

croissance, est déjà utilisée comme mécanisme d'amélioration de l'efficacité. Toutefois, la sélection génétique a été incapable à elle seule de répondre à toutes les attentes et a parfois entraîné des effets secondaires indésirables, comme une réduction de la longévité ou une sensibilité aux maladies. De plus, ces conséquences ont été aggravées par l'abandon des antibiotiques en traitements prophylactiques. Ce phénomène est particulièrement évident quand les animaux sont victimes d'un stress métabolique, comme pendant la transition chez les vaches laitières, la gestation chez les cochettes et la période qui suit le sevrage chez les porcelets. Au cours de ces épisodes, le déficit énergétique coïncide avec des réponses immunitaires dysfonctionnelles, une réaction inflammatoire incontrôlée et des conditions de stress oxydatif qui sont associées aux infections et maladies. Les preuves s'accumulent pour montrer que la mitochondrie, la principale source d'énergie de la cellule, est associée de près au stress oxydatif, à la réponse immunitaire, à l'inflammation et à la pathogenèse bactérienne. Détail intéressant, les fonctions de la mitochondrie sont assez sensibles à la quantité et à la variété de nutriments dans la ration. La récente reconnaissance de l'aptitude des nutriments à moduler certains mécanismes à la base des fonctions physiologiques a déclenché une révolution dans le domaine de la nutrition et a favorisé la réalisation de nombreuses études en nutrigénomique. Les approches de nutrigénomique axées sur la mitochondrie devraient pouvoir montrer que non seulement certains nutriments sont essentiels, mais aussi que des quantités spécifiques de nutriments en particulier sont nécessaires pour optimiser la santé, la résistance et la longévité des porcs et des vaches.

Introduction

The continuous increase in food demands is putting a constant pressure on livestock agriculture and the dairy and swine industries will have to considerably increase their production efficiency to remain competitive and economically profitable in coming decades. The productivity and value of commercial animals is determined by a combination of environmental and genetic factors. Genetic selection for specific trait characters such as milk production, prolificacy or animal growth is already being widely implemented as a mechanism to achieve increased efficiency. However, genetic selection by itself was found unable to answer all producers' expectations and, in some case, was accompanied by undesired side effects such as decrease in conception rates, reduction of longevity and susceptibility to highly problematic diseases. Moreover, these consequences were amplified by the actual withdrawal of antibiotics as prophylactic treatments. Antibiotics have been extensively used as growth promoters and/or as prophylactic treatment to prevent disease in the last decades in dairy and swine production (Barton 2014). However, there are increasing concerns in relation to the development of antibiotic-resistant (AR) bacterial strains and the potential of these and associated resistance genes to impact on animal and human health in many countries including Canada (Sheikh, Checkley et al. 2012, Durso and Cook 2014). As a consequence, many countries have taken or are starting to take action to control and reduce antibiotics usage in swine production (Barton 2014). However, such withdrawal of antibiotics and other restrictions have not yet been related to a reduction in total antimicrobial use or to a decrease in the resistance in key zoonotic bacteria. Indeed, the elimination of the prophylactic use of antibiotics was rather associated with an increase in health problems for the animals which require therapeutic use of antibiotics. The use

of antibiotics has become common practice in many countries to treat various animal health problems.

This situation is especially evident when animals encounter metabolic stresses such as throughout the transition period for dairy cows, the gestation for gilts and the post-weaning period for piglets (Jensen and Hayes 2014). This paper reviews the impact of negative energy balance in transition dairy cows, reproductive sows and post-weaned piglets as well as its association with occurrence of diseases and low productivity. It is now well recognized that negative energy balance is tightly related to major risk factors for health such as oxidative stress, inflammation, impairment of the immune response and susceptibility to pathogens and diseases. In all cells, mitochondria are remarkably dynamic organelles that are mainly known as the primary energy-generating system in the form of adenosine triphosphate (ATP) (Green and Tzagoloff 1966). Mitochondrial energy metabolism is however tightly associated with the production of toxic reactive oxygen species (ROS) that are susceptible to induce oxidative stress conditions in period of high metabolic activity (Kowaltowski, de Souza-Pinto et al. 2009). Mounting evidence indicates that mitochondrial function and oxidative stress are closely related to innate immune response (West, Shadel et al. 2011), inflammation (Circu and Aw 2012, López-Armada, Riveiro-Naveira et al. 2013), programmed cell death (Circu and Aw 2010) , and bacterial pathogenesis (Arnoult, Carneiro et al. 2009).

As a result, mitochondria from metabolically stressed animals require optimal conditions in term of antioxidant protection and metabolic substrates availability in order to ensure adequate energetic status, gut health and resistance to diseases. Interestingly, mitochondria are known to be quite sensitive to the quantity and variety of nutrients provided in diet (Baltzer, Tiefenböck et al. 2010, Schiff, Bénit et al. 2011). Thus, the main objectives of this paper are to describe how the mitochondria could be related to the numerous health issues associated to transition cows, reproductive sows and post-weaned piglets and to demonstrate how novel targeted nutrigenomics approaches specifically designed to support mitochondrial function could maximize productivity and profitability in dairy and swine production.

Mitochondria and Energy Production

Mitochondria are located at the interface between the environmental calorie supply and the energy requirements of each organ. Indeed, in response to energy demands, various substrates such as carbohydrates, proteins and fatty acids are metabolized via several pathways including glycolysis, β-oxidation, the tricarboxylic acid (TCA) or Krebs cycle and electron transport through the respiratory chain to ultimately drive energy synthesis, in the form of ATP, by oxidative phosphorylation (OXPHOS) (Green and Tzagoloff 1966). The mitochondria are the most efficient source of cellular ATP. The OXPHOS process involves the action of the mitochondrial respiratory chain consisting of five complexes located at the inner mitochondrial membrane level (Figure 1). The reduced form of nicotinamide-adenine dinucleotide (NADH) generated by the TCA cycle is initially oxidized by complex I. As the electrons from NADH are passed to the first mobile electron acceptor, oxidized coenzyme Q_{10} (CoQ_{10}) or ubiquinone, the energy is converted by the pumping of protons from the mitochondrial matrix toward the intermembrane space. CoQ_{10} can also accept electrons from complex II which have been donated

by the reduced form of flavin-adenine dinucleotide ($FADH_2$), another product of the TCA cycle. CoQ_{10} then passes electrons to complex III. Electrons are then transferred to the second mobile element in the respiratory chain, cytochrome c, which reduces complex IV that will ultimately drives the reduction of molecular oxygen to form water. This final dissipation of the redox energy at the level of complex IV is also associated with a final ejection of protons from the matrix. The passage of protons into the intermembrane space creates an electrochemical gradient that eventually drives the phosphorylation of ADP to ATP through complex V or ATPase when protons re-enter the matrix. The ATP is then transferred out of the mitochondria by the adenine nucleotide translocase complex and the energy became available for all cellular processes (Figure 1). Mitochondria that fail to generate a mitochondrial membrane potential and produce energy are targeted for destruction through mitophagy process. Significant correlation between expression levels of mitochondrial proteins implicated in energy metabolism and productivity have been recently observed in livestock (Grubbs, Fritchen et al. 2013). Thus, when metabolic demands are elevated as it is for modern dairy cows and pigs, the mitochondrial respiratory chain is heavily solicited for answering all energetic needs by generating large amount of ATP. The physiologic state of an animal determines the nutrient requirements and energetic efficiency of mitochondrial function. Numerous factors, such as high metabolic activity and hypoxia can significantly affect substrate utilization and activities of key mitochondrial enzymes.

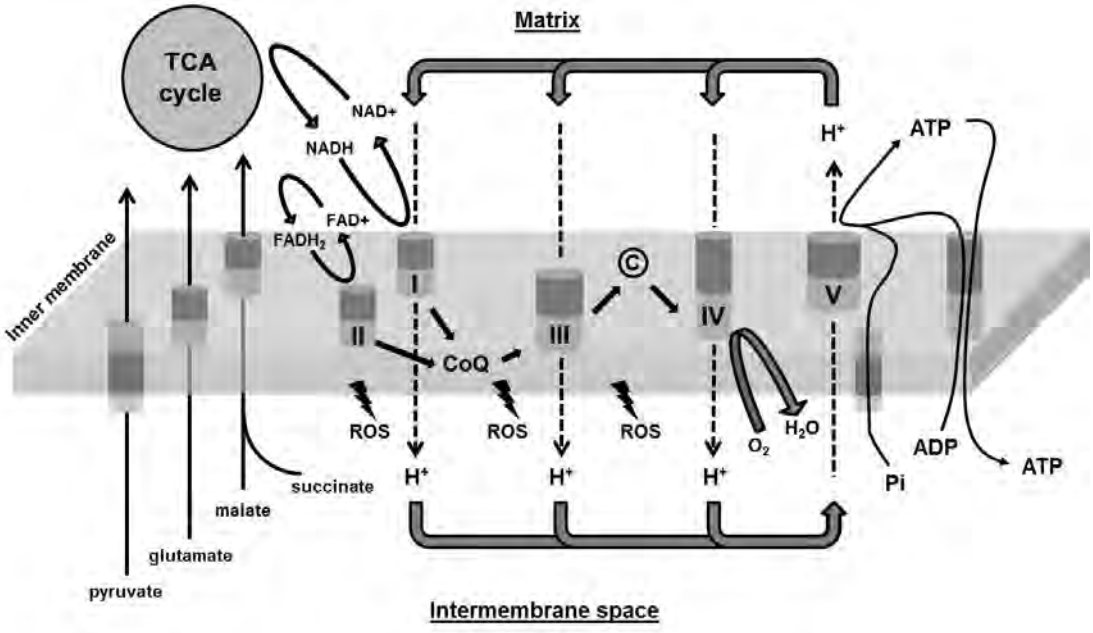


Figure 1. Schematic representation of the mitochondrial electron transport chain and oxydative phosphorylation (OXPHOS) system of energy production (Lapointe 2014). TCA cycle, tricarboxylic acid cycle; I to V, mitochondrial complexes I to V; CoQ, coenzyme Q or ubiquinone; (c), cytochrome C; H^+, protons; Pi, phosphate; ADP, adenosine diphosphate; ATP, adenosine triphosphate; ROS, reactive oxygen species; O_2, oxygen; H_2O, water; NAD, nicotinamide adenine dinucleotide oxidized or reduced (NADH); FAD, flavin adenine dinucleotide oxidized or reduced ($FADH_2$).

Reactive Oxygen Species (ROS): Toxic Side-products of Mitochondrial Energy Metabolism

ROS, including free radicals, are mainly generated as normal by-products of aerobic respiration and energy metabolism by mitochondria (Figure 1). Oxygen reduction during mitochondrial electron transport is the main source of the superoxide radical ($O_2^{\bullet -}$), which mitochondrial superoxide dismutases (SODs) turn into hydrogen peroxide (H_2O_2) and O_2. Importantly, because H_2O_2 is relatively stable and membrane-permeable, it can diffuse out of the mitochondria into the cytoplasm (Veal, Day et al. 2007). ROS can thus inflict serious damage to both mitochondrial and cytoplasmic macromolecules, such as lipids, nucleic acids, and proteins. Polyunsaturated fatty acids are one of the most sensitive oxidation targets for ROS because once lipid peroxidation is initiated, a damaging chain reaction takes place (Niki 2009). DNA bases are also very susceptible to ROS attack, and oxidation of DNA bases is believed to cause mutations and deletions in both nuclear and mitochondrial genomes (Fraga, Shigenaga et al. 1990). Almost all amino acid residues in a protein can be oxidized by ROS, with these modifications leading to losses of function (Ugarte, Petropoulos et al. 2010). Exposure to ROS appears to be unavoidable for cells living in an aerobic environment, and ROS toxicity is controlled by a complex network of non-enzymatic and enzymatic antioxidants, including the superoxide dismutases (SODs), the glutathione peroxidases (GPxs), the thioredoxin reductases (TRxs), the peroxiredoxins (PRxs), catalase, and glutathione (GSH) (Yu 1994, Flohe 2010). Therefore, oxidative stress can be defined as any imbalance between the production and the detoxification of ROS. Therefore, in periods of high energetic demands, the production of ROS by heavily solicited mitochondria largely exceeds the antioxidant potential, leading to oxidative stress conditions and inducing a wide range of oxidative damage in major cell structures (Balaban, Nemoto et al. 2005). It is well established that reproductive performance, resistance to diseases, immune response and longevity could all be negatively affected by mitochondrial oxidative stress in mammals (Agarwal, Gupta et al. 2006). The links between oxidative stress and development of adverse physiological outcomes constitute important issues in animal science and especially in metabolically stressed animals (Figure 2).

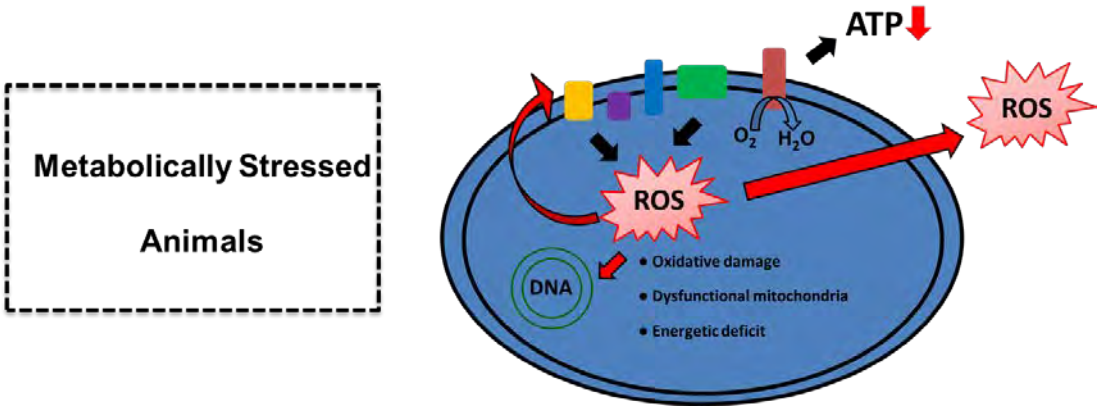


Figure 2. Schematic representation of the mitochondrial function and associated ROS production in metabolically stressed animals. When metabolic demands are elevated as it is for modern dairy cows and pigs, the mitochondrial respiratory chain is heavily solicited for answering all energetic needs by generating large amount of ATP. Mitochondrial energy production is associated with ROS production and high producing transition cows, hyperprolific sows and post-weaned piglets should handle substantive ROS amounts that are susceptible to induce oxidative damage and likely perturb reproductive processes, increase their vulnerability to various diseases and decrease their longevity

Mitochondria as Major Regulators of Reproduction, Immunity, Inflammation and Disease Resistance

Mounting evidence indicates that mitochondrial function and oxidative stress are closely related to innate immune response (West et al. 2011), intestinal inflammation (Circu and Aw 2012; López-Armada et al. 2013), reproduction (Agarwal, Gupta et al. 2006) and bacterial pathogenesis (Arnoult et al. 2009) (Figure 3). The toxicity of mitochondrial ROS is only one aspect of their action in living cells as ROS originating from mitochondria and other cellular sites can also modulate the function of various signalling pathways. In fact under physiological and stress conditions, the transient generation of ROS, within boundaries, appears to be essential to maintain cellular homeostasis.

Similarly to other physiological processes, a minimal amount of ROS is crucial to achieve good reproduction performance. In females, this implication is broad and is related to almost all reproductive events including cyclic luteal and endometrial changes, follicular development, ovulation, fertilization, embryogenesis, embryonic implantation, placental differentiation and labour (Fujii, Iuchi et al. 2005, Agarwal, Gupta et al. 2008). Age-related decline in female fertility is a well-documented phenomenon which in most species occurs long before death. Ovarian dysfunction account for most of this loss of reproductive function and is characterized by declines in ovarian follicle numbers and in oocyte quality (Lim and Luderer 2011). Several studies now suggest that mitochondrial oxidative stress may play a role in the age-related decline in ovarian function (Navarro, Torrejon et al. 2005, Ramalho-Santos, Varum et al. 2009). Mitochondrial ROS are also the major host defense mechanism against infection and harmful agents, and mitochondria are now recognized as major mediators of inflammation. It has been

shown that pro-inflammatory factors impair mitochondrial activity and the resulting mitochondrial dysfunction could modulate inflammatory responses through both redox-sensitive inflammatory pathways and direct activation of the inflammasome (López-Armada, Riveiro-Naveira et al. 2013). Mitochondria could thus integrate these 2 pathways, leading to an overstimulation of the inflammatory response. A decline in mitochondrial function is essential to the development of the inflammatory phenotype observed in degenerative or acute diseases as well as in advanced age. Mitochondria are now considered as central hubs of immune-cell regulation. Many studies have revealed that in immune cells, mitochondria participate in signaling through ROS production, metabolite availability, and by physically acting as scaffolding for protein interaction (West, Shadel et al. 2011, Weinberg, Sena et al. 2015). Mitochondrial signals appear to be necessary for the immune cell to fulfill its specific role in the immune response in both innate and adaptive settings to a variety of intruders. Mitochondrial signaling dictates macrophage polarization and function, regulates T cell activation and controls CD8+ memory T cell formation (Leavy 2013).

Mitochondria have also been identified as the target of an increasing number of bacterial proteins that are transferred to the cell by virulent bacteria such as enterotoxigenic *E. coli* (ETEC) and *Salmonella* (*S. enterica*) (Rudel, Kepp et al. 2010, Jiang, Tong et al. 2012, Hicks and Galán 2013). These pathogens have developed multiple strategies to subvert the cell death machinery of the host, including mitochondria-mediated mechanisms, with the purpose of (i) avoiding the premature death of infected cells, to facilitate intracellular replication; (ii) killing infected cells at a late stage of the microbial life cycle, to favor pathogen dissemination; or (iii) destroying uninfected cell of the immune system, to subvert anti-microbial control. These bacteria are problematic during the post-weaning period in piglets, leading to severe loss of mitochondrial energy production and disruption of the epithelial barrier function. This results in substantial losses per annum through mortality, cost of medication and growth reduction (Sargeant, Miller et al. 2011, Miarelli, Drumo et al. 2016).

Mitochondria as Promising Nutritional Targets in Dairy and Swine Production

1) Transition Dairy Cows

Dairy cows encounter severe metabolic stresses during the traumatic period surrounding calving, known as the transition period, as feed intake is too low to meet energy requirements for maintenance and milk production (LeBlanc 2010). To meet these requirements, high-yielding dairy cows mobilize body fat resulting in an augmented hepatic mitochondrial oxidative metabolism (Schaff, Borner et al. 2012). This energetic deficit coincides with dysfunctional immune responses, uncontrolled inflammation and acute oxidative stress conditions which are tightly associated with infections and diseases (Sordillo and Aitken 2009, Sordillo, Contreras et al. 2009). Most of the metabolic diseases of dairy cows such as milk fever, ketosis, fatty liver, insulin resistance, hypocalcaemia and retained placenta occur within the first two weeks of lactation. Moreover the etiology of many metabolic diseases that are not clinically apparent during this problematic period can be traced back to insults that occurred during transition period (Wathes 2012). It is also well known that the majority of infectious diseases, like mastitis and

metritis, are mainly affecting transition cows. Thus, it is not surprising to observe that the transition period is characterized by an intensive use of antibiotics.

Although the transition period is associated with a peak incidence of metabolic and infectious diseases, the effects of these diseases on dairy cow health and productivity extend far into the following lactation (Mulligan and Doherty 2008). Additionally, during lactation, both the development and the metabolic rate of the mammary gland undergo a biphasic change that is associated with an increase in rate of milk synthesis. In mammary tissue, the rate of oxygen consumption increases dramatically with the onset of lactation and a significant increase in secretory cell mitochondrial number was observed (Hadsell, Torres et al. 2006). These observations support the hypothesis that during lactation, secretory epithelial cells within the mammary gland are exposed to increasing amounts of toxic reactive oxygen species produced by mitochondria resulting in oxidative damage and programmed cell death. Taken together, these observations suggest that managing strategies for dairy cows during the transition period should be geared toward reducing negative energy balance by feeding specially formulated diets to improve mitochondrial function.

2) Gestating and Lactating Sows

Breeding herd productivity is the primary factor that determines pork industry profitability. Indeed, large litters of healthy piglets delivered by highly productive sows that breed regularly with minimal culling rate provide the best scenario for long-term economic survivability of producers. The productivity of sows is generally determined by a combination of environmental and genetic factors. In this optic, genetic selection and management changes during the last decades have increased the average litter size of sows in many countries including Canada (Foxcroft 2012). Thus, the producers aim to conserve these genetically selected proliferative sows for several parities in order to maximize their production and enhance their benefits (Lucia, Dial et al. 2000). The longevity or lifetime production of a sow is normally defined as the number of pigs weaned per sow per lifetime and it was established that a sow must produce at least 4 to 6 litters for optimal economics (Serenius and Stalder 2004; Rodriguez-Zas, Davis et al. 2006).

Unfortunately, the recent success that was obtained in term of increasing litter size has not correlated with an extension of reproductive sow longevity and reduction of replacement rate. Indeed, it has been estimated that as much as 40 to 50% of sows are culled annually with over one-third of these removals attributed to reproduction problems (Rodriguez-Zas, Southey et al. 2003). Moreover, roughly half of these culls attributed to reproduction failure are associated with replacement gilts (Engblom, Lundeheim et al. 2008). In contrast to multiparous sows, gilts have not yet reached the optimal body composition in term of fat tissue mass that is required to adequately support pregnancy and lactation (Quesnel, Etienne et al. 2007, Hoge and Bates 2010, Schenkel, Bernardi et al. 2010). Gilts from modern genetic lines are much leaner than their counterparts of previous decades and are therefore more vulnerable to energetic and metabolic deficiencies (Cottney, Magowan et al. 2012, Hoving, Soede et al. 2012, Roongsitthichai, Cheuchuchart et al. 2013). Such problems associated with gilts are costly to producers and there is thus an increased economic demand for gilts with greater reproductive potential, resistance and longevity (Hoge and Bates 2010, Onteru, Fan et al. 2011, Knauer, Cassady et al. 2012).

Considering that the maintenance of the proper number of females in each breeding group depends upon the introduction of replacement gilts, it is crucial for producers that those animals reproduce efficiently and there is thus an increased economic demand for gilts with greater reproductive potential, resistance and longevity (Onteru, Fan et al. 2011). It is also well known that litter size generally increases with each parity beyond the second litter before starting to drastically decline after 6 parities (Quesnel, Brossard et al. 2008).

Reproductive sows are known to have high energetic demands associated with growth and energy utilization during critical periods such as gestation and lactation, which may have an impact on reproductive and litter trait performances (Willis, Zak et al. 2003). Given the increased in litter size observed in the last decades, aged sows and first parity gilts frequently fail to satisfy their energetic nutrient requirements even if voluntary feed intake usually increases with litter size (Quesnel, Etienne et al. 2007). As a consequence, sows nursing large litters lose generally more body weight during lactation than sows nursing smaller litters. Thus, in order to adequately fulfill their cellular energetic needs throughout gestation and lactation processes, hyperprolific sows completely rely on the maintenance of functional mitochondria (Lapointe 2014). Recent results from our laboratory further indicate that replacement gilts sustain significantly higher oxidative conditions than multiparous sows. These findings may contribute to the design of nutritional regimens that will increase the productivity of gilts and sows by improving mitochondrial function and counteracting oxidative stress.

3) Post-Weaned Piglets

Weaning is known to impose tremendous stress on piglets and the period following weaning is characterised by a high incidence of intestinal disturbances, bacterial infections and energetic deficiencies that led to serious diseases (Pie, Lalles et al. 2004, Lalles, Bosi et al. 2007, Wijtten, van der Meulen et al. 2011). Unfortunately this problem was exacerbated by the selection for hyperprolific sows as a means of increasing litter size and profit which has resulted in more pronounced variations of within-litter birth weights as well as in an increased number of low-birth weight (LBW) piglets (Damgaard, Rydhmer et al. 2003). These LBW piglets are more fragile to intestinal infectious diseases and energetic deficiencies (De Vos, Che et al. 2014). Recent evidence suggests that oxidative stress negatively affects the integrity of intestinal barrier function (John, Fromm et al. 2011). The precarious health condition of post-weaned piglets has been associated to intense utilisation of antibiotics as prophylactic treatments in swine production in attempt to limit the occurrence of diseases. However, the current withdrawal of antibiotics and other restrictions adopted by several countries was associated with an increase in health problems among the newly weaned piglets which require therapeutic use of antibiotics (Jensen & Hayes, 2014). As a result, effective strategies aiming to improve metabolic health of piglets and increase their defense mechanisms against bacterial infections need to be developed to limit antibiotic use in swine production and ensure profitability. Moreover, it is evidence that mitochondria from newly weaned piglets require optimal conditions in term of antioxidant protection and metabolic substrates availability in order to ensure adequate energetic status, gut health and resistance to diseases. However, even if such concept is highly promising, no corresponding targeted nutritional strategy is actually available for producers.

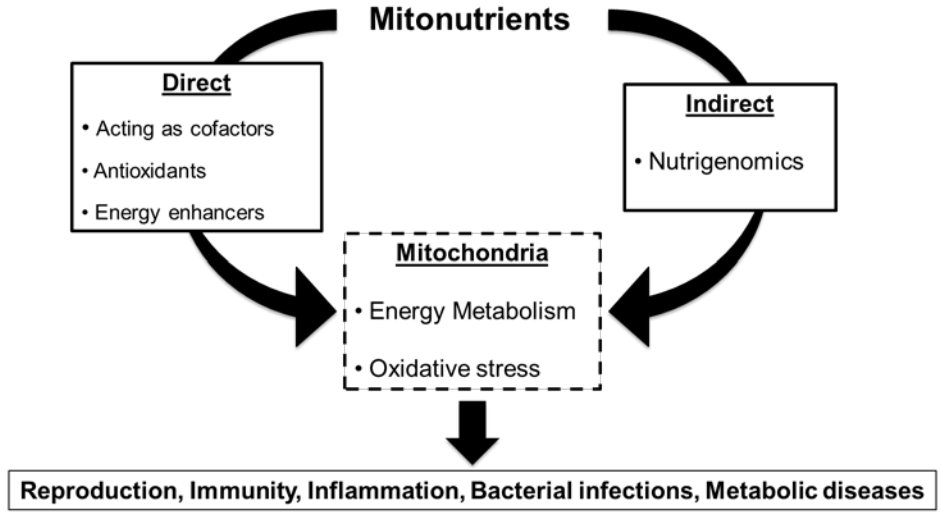


Figure 3. Schematic representation of the potential effects of a targeted nutritional intervention with mitonutrients on mitochondrial function and the impact on physiological processes. Targeted nutritional interventions with mitonutrients can be beneficial in preventing and improving mitochondrial function mainly by stimulating mitochondrial energy production and by decreasing oxidative stress. Those nutrients could exert their function either directly by being actively implicated in specific mitochondrial biochemical pathways or indirectly by enhancing the expression of genes (nutrigenomics) encoding proteins involved in mitochondrial biology. The mitonutrients will ultimately impact many physiological processes and occurrence of diseases known to be linked to mitochondrial function and oxidative stress.

Optimizing Mitochondrial Function with "Mitonutrients"

As mentioned earlier, mitochondrial energy production is tightly associated with ROS production and high producing transition cows, hyperprolific gilts and post-weaned piglets should handle substantive ROS amounts that are susceptible to induce oxidative damage and likely perturb reproductive processes, increase their vulnerability to various diseases and decrease their longevity (Jansen and Burton 2004, Pieczenik and Neustadt 2007). As a result, their mitochondria require optimal conditions in term of antioxidant protection and metabolic substrates availability in order to ensure reproductive success, health and long-term productivity (Figure 3).

Such aim to optimize mitochondrial function could be seen as ambitious and relatively complex but these organelles are known to be quite sensitive to the quantity and variety of nutrients provided in diet. Variation in protein content has a major effect on mitochondrial number and also specifically affects activities of numerous mitochondrial enzymes. Specific change in carbohydrate diet has a large effect on enzymes involved in energy metabolism, especially those related to triglyceride synthesis, and can affect mitochondrial function through changes in mitochondrial phospholipid composition (Wander and Berdanier 1985). Accordingly, several

vitamin and mineral deficiencies can result in aberrant expression of mitochondrial proteins and have a deleterious effect on mitochondrial structure, biogenesis and function (Aw and Jones 1989). These particular phenotypes can be frequently reversed by nutrient repletion, which suggests that nutritional supplementation may be useful to support mitochondrial function in stressful conditions. Interestingly, increasing evidence now suggests that targeting mitochondria with specific nutrients from natural sources, now termed mitochondrial nutrients or "mitonutrients", could efficiently prevent and ameliorate various conditions associated with mitochondrial dysfunction. Several drugs are also continuously characterized for their positive actions on mitochondria but this paper will strictly focus on natural nutrients which have greater potential of being used in livestock's diet in a near future. Recent studies have shown that targeted nutritional interventions with mitonutrients can be beneficial in preventing and improving mitochondrial function mainly by stimulating mitochondrial energy production, enhancing mitochondrial metabolism (biogenesis and degradation) as well as by decreasing oxidative stress. Those nutrients could exert their function either directly by being actively implicated in specific mitochondrial biochemical pathways or indirectly by enhancing the expression of genes encoding proteins involved in mitochondrial biology (Baltzer, Tiefenböck et al. 2010). In other terms, mitonutrients could be incorporated and being immediately active in mitochondria or having delayed nutrigenomic effects (Figure 3). Nutrigenomics has evolved to signify the field concerned by the investigation of the effects of nutrients on gene expression and related downstream molecular and biological events. The objective of nutrigenomics research is to study the study genome-wide influences of nutrition on metabolic stress in the genesis of metabolic syndrome and the prevention of diseases (Afman and Müller 2006, Bouchard and Ordovas 2012). This section is dedicated to the enumeration and basic description of the mitochondrial actions of several recognized mitonutrients that are susceptible of being of interest for the elaboration of nutritional strategies in swine and dairy production (Figure 3). Particular emphasis will be placed on their potential to serve as alternative energy sources, enhance mitochondrial antioxidant defense system, induce mitochondrial biogenesis or improve mitochondrial function by acting as cofactors in biochemical processes.

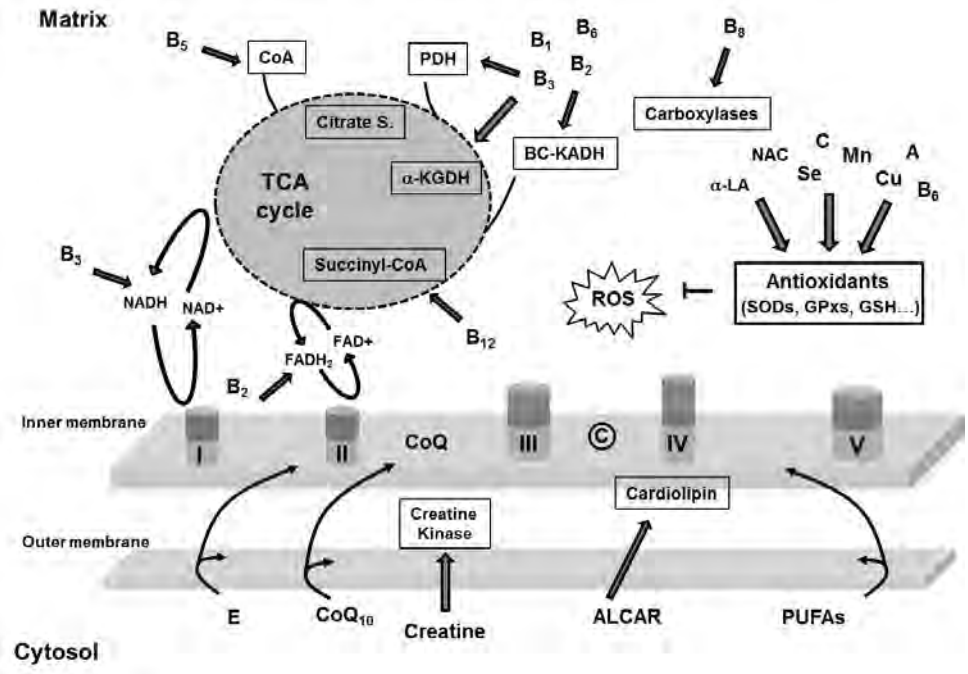


Figure 4. Targets and modes of action of mitonutrients (Lapointe 2014). TCA cycle, Tricarboxylic acid cycle; I to V, Mitochondrial complexes I to V; NAD, Nicotinamide adenine dinucleotide oxidized or reduced (NADH); FAD, Flavin adenine dinucleotide oxidized or reduced (FADH2); Citate S., Citrate synthase; CoA, Coenzyme A; PDH, Pyruvate dehydrogenase; α-KGDH, Alpha-ketoglutarate dehydrogenase; BC-KADH, Branched chain ketoacid dehydrogenase, SODs, Superoxide dismutases, GPxs, Glutathione peroxidases; GSH, Glutathione; ROS, reactive oxygen species; CoQ, coenzyme Q or ubiquinone; (c), Cytochrome C; B_1 to B_{12}, B vitamins; E, vitamin E; C, vitamin C; a-LA, Alpha-lipoic acid; NAC, N-acetylcysteine; Se, Selenium; Mn, Manganese; Cu; Copper; ALCAR, Acetyl-L-carnitine; PUFAs, polyunsaturated fatty acids.

Several nutrients with antioxidant properties such as α-lipoic acid, coenzyme Q10 (CoQ_{10}), vitamin E, selenium and vitamin B2 have been shown to positively affect mitochondrial function. Alpha lipoic acid (α-LA) is a powerful mitochondrial antioxidant and a coenzyme found naturally in mitochondria and involved in energy metabolism. Dietary supplemented α-LA is known to exert an anti-inflammatory action within cells (Koriyama, Nakayama et al. 2013). Coenzyme Q_{10} is a bioactive lipid which is principally known as an electron carrier in the mitochondrial respiratory chain. It is also recognized for its antioxidant, anti-apoptotic and anti-inflammatory properties and is incorporated within mitochondrial membranes when used as a nutritional supplement (Bentinger, Tekle et al. 2010, Lapointe, Wang et al. 2012). Vitamin E is a lipid soluble antioxidant presents in mitochondrial membranes (Lauridsen and Jensen 2012). Selenium (Se) is a mineral that participates in the synthesis of selenoproteins with strong antioxidant properties, such as glutathione peroxidases (GPxs) and thioredoxin reductases (TRxs) (Dursun, Taskin et al. 2011). B vitamins are especially important for supporting mitochondrial function because they directly act as cofactors for mitochondrial enzymes or as precursors of important cofactors (Figure 4). It is well recognized that B vitamins play an

essential role in mitochondrial aerobic respiration and energy production. Furthermore, mitochondrial integrity and functions are compromised by dietary deficiency of B vitamins (Depeint, Bruce et al. 2006). Riboflavin (vitamin B2) is a water soluble vitamin that is the major component of the flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN), which function as redox cofactors in the mitochondrial respiratory chain (Depeint, Bruce et al. 2006). Other mitochondrial nutrients such as creatine and L-carnitine have the capacity to act as energetic enhancers. Creatine is actively incorporated in mitochondria to act as an energy-boosting compound by increasing creatine/phosphocreatine stores and consequently preventing ATP depletion. L-carnitine and its acetyl derivative (Acetyl-L-carnitine; ALCAR) transport long-chain fatty acids into mitochondria for β-oxidation and production. Furthermore, it was shown that some combinations of nutrients may possess unique functions and be more efficient than the individual nutrients. Several studies thus indicate that the antioxidant and/or the energetic potential of CoQ_{10} could be more efficient when acting with either α-tocopherol, α-lipoic acid or creatine (Marriage, Clandinin et al. 2003). Similarly, it was shown that a combination of α-lipoic acid and L-carnitine significantly improve mitochondrial function and stimulate mitochondrial biogenesis (Tarnopolsky 2008). These strong synergistic effects between mitochondrial nutrients could be observed both in cell/tissue culture and at the whole animal level (Figure 3).

Conclusion and Perspectives

The numerous benefits of mitochondrial targeted nutrients on animal health have been demonstrated in many organ systems via their ability to improve mitochondrial function and decrease oxidative damage. Of particular interest, the promising results obtained with defined combinations of nutrients with complementary and synergistic effects now provide great expectations for preventing and treating physiological problems associated with mitochondrial dysfunction such as susceptibility to diseases and infections, reproductive disorders and poor longevity. The impact of many mitochondrial nutrients on mitochondrial function is yet to be fully appreciated in swine and dairy production but the benefits shown in supplementation of those nutrients in other animals as well as in vitro experiments seem more than sufficient to encourage further studies. Therefore, the future directions should include the use of modern technology of nutrigenomics to identify novel combinations of precise mitochondrial nutrients for optimal effects on mitochondrial energy metabolism, oxidative stress and immune system. Investigating the mechanisms of action of mitochondrial nutrients at the cellular and molecular levels should also greatly help in the elaboration and the fine tuning of such novel nutritional interventions. While more research is definitely needed to investigate the safety and efficacy of mitochondrial nutrients in improving performance of dairy cows, reproductive sows and weaned piglets, this could represents a promising area of interests for producers looking for long-term productivity and profitability of their herds.

References

Afman, L. and M. Müller (2006). "Nutrigenomics: From Molecular Nutrition to Prevention of Disease." Journal of the American Dietetic Association **106**(4): 569-576.

Agarwal, A., S. Gupta, L. Sekhon and R. Shah (2008). "Redox considerations in female reproductive function and assisted reproduction: from molecular mechanisms to health implications." Antioxidants & redox signaling **10**(8): 1375-1403.

Agarwal, A., S. Gupta and S. Sikka (2006). "The role of free radicals and antioxidants in reproduction." Current opinion in obstetrics & gynecology **18**(3): 325-332.

Arnoult, D., L. Carneiro, I. Tattoli and S. E. Girardin (2009). "The role of mitochondria in cellular defense against microbial infection." Seminars in Immunology **21**(4): 223-232.

Aw, T. Y. and D. P. Jones (1989). "Nutrient supply and mitochondrial function." Annu Rev Nutr **9**: 229-251.

Balaban, R. S., S. Nemoto and T. Finkel (2005). "Mitochondria, oxidants, and aging." Cell **120**(4): 483-495.

Baltzer, C., S. K. Tiefenböck and C. Frei (2010). "Mitochondria in response to nutrients and nutrient-sensitive pathways." Mitochondrion **10**(6): 589-597.

Barton, M. D. (2014). "Impact of antibiotic use in the swine industry." Curr Opin Microbiol **19**: 9-15.

Bentinger, M., M. Tekle and G. Dallner (2010). "Coenzyme Q--biosynthesis and functions." Biochemical and biophysical research communications **396**(1): 74-79.

Bouchard, C. and J. M. Ordovas (2012). "Fundamentals of Nutrigenetics and Nutrigenomics." **108**: 1-15.

Circu, M. L. and T. Y. Aw (2010). "Reactive oxygen species, cellular redox systems, and apoptosis." Free Radic Biol Med **48**(6): 749-762.

Circu, M. L. and T. Y. Aw (2012). "Intestinal redox biology and oxidative stress." Seminars in Cell & Developmental Biology **23**(7): 729-737.

Cottney, P. D., E. Magowan, M. E. E. Ball and A. Gordon (2012). "Effect of oestrus number of nulliparous sows at first service on first litter and lifetime performance." Livestock Science **146**(1): 5-12.

Damgaard, L. H., L. Rydhmer, P. Lovendahl and K. Grandinson (2003). "Genetic parameters for within-litter variation in piglet birth weight and change in within-litter variation during suckling." J Anim Sci **81**(3): 604-610.

De Vos, M., L. Che, V. Huygelen, S. Willemen, J. Michiels, S. Van Cruchten and C. Van Ginneken (2014). "Nutritional interventions to prevent and rear low-birthweight piglets." J Anim Physiol Anim Nutr (Berl) **98**(4): 609-619.

Depeint, F., W. R. Bruce, N. Shangari, R. Mehta and P. J. O'Brien (2006). "Mitochondrial function and toxicity: Role of the B vitamin family on mitochondrial energy metabolism." Chemico-Biological Interactions **163**(1-2): 94-112.

Durso, L. M. and K. L. Cook (2014). "Impacts of antibiotic use in agriculture: what are the benefits and risks?" Curr Opin Microbiol **19**: 37-44.

Dursun, N., E. Taskin, M. B. Yerer Aycan and L. Sahin (2011). "Selenium-mediated cardioprotection against adriamycin-induced mitochondrial damage." Drug and chemical toxicology **34**(2): 199-207.

Flohe, L. (2010). "Changing paradigms in thiology from antioxidant defense toward redox regulation." Methods Enzymol **473**: 1-39.

Fraga, C. G., M. K. Shigenaga, J. W. Park, P. Degan and B. N. Ames (1990). "Oxidative damage to DNA during aging: 8-hydroxy-2'-deoxyguanosine in rat organ DNA and urine." Proc Natl Acad Sci U S A **87**(12): 4533-4537.
Fujii, J., Y. Iuchi and F. Okada (2005). "Fundamental roles of reactive oxygen species and protective mechanisms in the female reproductive system." Reproductive biology and endocrinology : RB&E **3**: 43.
Green, D. E. and A. Tzagoloff (1966). "The mitochondrial electron transfer chain." Archives of biochemistry and biophysics **116**(1): 293-304.
Grubbs, J. K., A. N. Fritchen, E. Huff-Lonergan, N. K. Gabler and S. M. Lonergan (2013). "Selection for residual feed intake alters the mitochondria protein profile in pigs." Journal of Proteomics **80**: 334-345.
Hadsell, D., D. Torres, J. George, A. Capuco, S. Ellis and M. Fiorotto (2006). "Changes in secretory cell turnover, and mitochondrial oxidative damage in the mouse mammary gland during a single prolonged lactation cycle suggest the possibility of accelerated cellular aging." Experimental Gerontology **41**(3): 271-281.
Hicks, S. W. and J. E. Galán (2013). "Exploitation of eukaryotic subcellular targeting mechanisms by bacterial effectors." Nature Reviews Microbiology **11**(5): 316-326.
Hoge, M. D. and R. O. Bates (2010). "Developmental factors that influence sow longevity." Journal of Animal Science **89**(4): 1238-1245.
Hoving, L. L., N. M. Soede, H. Feitsma and B. Kemp (2012). "Lactation weight loss in primiparous sows: consequences for embryo survival and progesterone and relations with metabolic profiles." Reprod Domest Anim **47**(6): 1009-1016.
Jansen, R. P. and G. J. Burton (2004). "Mitochondrial dysfunction in reproduction." Mitochondrion **4**(5-6): 577-600.
Jensen, H. H. and D. J. Hayes (2014). "Impact of Denmark's ban on antimicrobials for growth promotion." Curr Opin Microbiol **19**: 30-36.
Jiang, J.-H., J. Tong and K. Gabriel (2012). "Hijacking Mitochondria: Bacterial Toxins that Modulate Mitochondrial Function." IUBMB Life **64**(5): 397-401.
John, L. J., M. Fromm and J. D. Schulzke (2011). "Epithelial barriers in intestinal inflammation." Antioxid Redox Signal **15**(5): 1255-1270.
Knauer, M. T., J. P. Cassady, D. W. Newcom and M. T. See (2012). "Gilt development traits associated with genetic line, diet and fertility." Livestock Science **148**(1-2): 159-167.
Koriyama, Y., Y. Nakayama, S. Matsugo, K. Sugitani, K. Ogai, T. Takadera and S. Kato (2013). "Anti-inflammatory effects of lipoic acid through inhibition of GSK-3β in lipopolysaccharide-induced BV-2 microglial cells." Neuroscience Research.
Kowaltowski, A. J., N. C. de Souza-Pinto, R. F. Castilho and A. E. Vercesi (2009). "Mitochondria and reactive oxygen species." Free Radic Biol Med **47**(4): 333-343.
Lalles, J. P., P. Bosi, H. Smidt and C. R. Stokes (2007). "Nutritional management of gut health in pigs around weaning." Proc Nutr Soc **66**(2): 260-268.
Lapointe, J. (2014). "Mitochondria as promising targets for nutritional interventions aiming to improve performance and longevity of sows." J Anim Physiol Anim Nutr (Berl) **98**(5): 809-821.
Lapointe, J., Y. Wang, E. Bigras and S. Hekimi (2012). "The submitochondrial distribution of ubiquinone affects respiration in long-lived Mclk1+/- mice." J Cell Biol **199**(2): 215-224.
Lauridsen, C. and S. K. Jensen (2012). "α-Tocopherol incorporation in mitochondria and microsomes upon supranutritional vitamin E supplementation." Genes & Nutrition **7**(4): 475-482.

Leavy, O. (2013). "T cells: Mitochondria and T cell activation." Nat Rev Immunol **13**(4): 224.
LeBlanc, S. (2010). "Monitoring metabolic health of dairy cattle in the transition period." J Reprod Dev **56 Suppl**: S29-35.
Lim, J. and U. Luderer (2011). "Oxidative damage increases and antioxidant gene expression decreases with aging in the mouse ovary." Biology of reproduction **84**(4): 775-782.
López-Armada, M. J., R. R. Riveiro-Naveira, C. Vaamonde-García and M. N. Valcárcel-Ares (2013). "Mitochondrial dysfunction and the inflammatory response." Mitochondrion **13**(2): 106-118.
Marriage, B., M. T. Clandinin and D. M. Glerum (2003). "Nutritional cofactor treatment in mitochondrial disorders." J Am Diet Assoc **103**(8): 1029-1038.
Miarelli, M., R. Drumo, F. Signorelli, C. Marchitelli, S. Pavone, M. Pesciaroli, J. Ruggieri, B. Chirullo, S. Ammendola, A. Battistoni, G. L. Alborali, E. Manuali and P. Pasquali (2016). "Salmonella Typhimurium infection primes a nutriprive mechanism in piglets." Vet Microbiol **186**: 117-125.
Mulligan, F. J. and M. L. Doherty (2008). "Production diseases of the transition cow." Vet J **176**(1): 3-9.
Navarro, A., R. Torrejon, M. J. Bandez, J. M. Lopez-Cepero and A. Boveris (2005). "Mitochondrial function and mitochondria-induced apoptosis in an overstimulated rat ovarian cycle." American journal of physiology. Endocrinology and metabolism **289**(6): E1101-1109.
Niki, E. (2009). "Lipid peroxidation: physiological levels and dual biological effects." Free Radic Biol Med **47**(5): 469-484.
Onteru, S. K., B. Fan, M. T. Nikkila, D. J. Garrick, K. J. Stalder and M. F. Rothschild (2011). "Whole-genome association analyses for lifetime reproductive traits in the pig." Journal of animal science **89**(4): 988-995.
Pie, S., J. P. Lalles, F. Blazy, J. Laffitte, B. Seve and I. P. Oswald (2004). "Weaning is associated with an upregulation of expression of inflammatory cytokines in the intestine of piglets." J Nutr **134**(3): 641-647.
Pieczenik, S. R. and J. Neustadt (2007). "Mitochondrial dysfunction and molecular pathways of disease." Experimental and molecular pathology **83**(1): 84-92.
Quesnel, H., M. Etienne and M. C. Pere (2007). "Influence of litter size on metabolic status and reproductive axis in primiparous sows." J Anim Sci **85**(1): 118-128.
Ramalho-Santos, J., S. Varum, S. Amaral, P. C. Mota, A. P. Sousa and A. Amaral (2009). "Mitochondrial functionality in reproduction: from gonads and gametes to embryos and embryonic stem cells." Human Reproduction Update **15**(5): 553-572.
Roongsitthichai, A., P. Cheuchuchart, S. Chatwijitkul, O. Chantarothai and P. Tummaruk (2013). "Influence of age at first estrus, body weight, and average daily gain of replacement gilts on their subsequent reproductive performance as sows." Livestock Science **151**(2-3): 238-245.
Rudel, T., O. Kepp and V. Kozjak-Pavlovic (2010). "Interactions between bacterial pathogens and mitochondrial cell death pathways." Nature Reviews Microbiology **8**(10): 693-705.
Sargeant, H. R., H. M. Miller and M. A. Shaw (2011). "Inflammatory response of porcine epithelial IPEC J2 cells to enterotoxigenic E. coli infection is modulated by zinc supplementation." Mol Immunol **48**(15-16): 2113-2121.
Schaff, C., S. Borner, S. Hacke, U. Kautzsch, D. Albrecht, H. M. Hammon, M. Rontgen and B. Kuhla (2012). "Increased anaplerosis, TCA cycling, and oxidative phosphorylation in the liver of dairy cows with intensive body fat mobilization during early lactation." J Proteome Res **11**(11): 5503-5514.

Schenkel, A. C., M. L. Bernardi, F. P. Bortolozzo and I. Wentz (2010). "Body reserve mobilization during lactation in first parity sows and its effect on second litter size." Livestock Science **132**(1-3): 165-172.
Schiff, M., P. Bénit, A. Coulibaly, S. Loublier, R. El-Khoury and P. Rustin (2011). "Mitochondrial response to controlled nutrition in health and disease." Nutrition Reviews **69**(2): 65-75.
Sheikh, A. A., S. Checkley, B. Avery, G. Chalmers, V. Bohaychuk, P. Boerlin, R. Reid-Smith and M. Aslam (2012). "Antimicrobial resistance and resistance genes in Escherichia coli isolated from retail meat purchased in Alberta, Canada." Foodborne Pathog Dis **9**(7): 625-631.
Sordillo, L. M. and S. L. Aitken (2009). "Impact of oxidative stress on the health and immune function of dairy cattle." Veterinary Immunology and Immunopathology **128**(1-3): 104-109.
Sordillo, L. M., G. A. Contreras and S. L. Aitken (2009). "Metabolic factors affecting the inflammatory response of periparturient dairy cows." Animal Health Research Reviews **10**(01): 53-63.
Tarnopolsky, M. A. (2008). "The mitochondrial cocktail: Rationale for combined nutraceutical therapy in mitochondrial cytopathies☆." Advanced Drug Delivery Reviews **60**(13-14): 1561-1567.
Ugarte, N., I. Petropoulos and B. Friguet (2010). "Oxidized mitochondrial protein degradation and repair in aging and oxidative stress." Antioxid Redox Signal **13**(4): 539-549.
Veal, E. A., A. M. Day and B. A. Morgan (2007). "Hydrogen peroxide sensing and signaling." Mol Cell **26**(1): 1-14.
Wander, R. C. and C. D. Berdanier (1985). "Effects of dietary carbohydrate on mitochondrial composition and function in two strains of rats." J Nutr **115**(2): 190-199.
Wathes, D. C. (2012). "Mechanisms Linking Metabolic Status and Disease with Reproductive Outcome in the Dairy Cow." Reproduction in Domestic Animals **47**: 304-312.
Weinberg, S. E., L. A. Sena and N. S. Chandel (2015). "Mitochondria in the regulation of innate and adaptive immunity." Immunity **42**(3): 406-417.
West, A. P., G. S. Shadel and S. Ghosh (2011). "Mitochondria in innate immune responses." Nature Reviews Immunology **11**(6): 389-402.
Wijtten, P. J., J. van der Meulen and M. W. Verstegen (2011). "Intestinal barrier function and absorption in pigs after weaning: a review." Br J Nutr **105**(7): 967-981.
Willis, H. J., L. J. Zak and G. R. Foxcroft (2003). "Duration of lactation, endocrine and metabolic state, and fertility of primiparous sows." Journal of animal science **81**(8): 2088-2102.
Yu, B. P. (1994). "Cellular defenses against damage from reactive oxygen species." Physiol Rev **74**(1): 139-162.

Consumers' Attitudes on Meat Production: What They Know, What They Don't Know and Why It Matters

Mike von Massow

Food, Agricultural and Resource Economics

Why talk about consumers at all?

- This has been a polarized debate
- Many Canadians not active in the discussion
  - Its who everyone is trying to influence
    - Not aware?
    - Don't care?
    - Not worried?
- They make purchase decisions
  - Establishing an acceptable standard for society
    - Not just about what they will buy

Food, Agricultural and Resource Economics

Polarized discussion

- Activists
  - We need to do better
  - Consumers care
- Industry
  - Consumers trust us
  - We are doing a good job
  - If you want more you need to pay us for it

Food, Agricultural and Resource Economics

Are antibiotics a concern?

- Some will argue that the level of awareness and concern is very high
  - Consumer Reports survey found 72% very or extremely concerned, connected to antibiotic resistance
  - Less publically availabe data on antibiotic use generally
    - Can we find clues elsewhere?

Food, Agricultural and Resource Economics

AW in the consciousness of Canadians

- 31% agreed/strongly agreed that their food choices have a large impact on the well-being of farm animals
- 58% agreed/strongly agreed that gov't should take a role in ensuring animal welfare
- 50% said labels should be clearer
- 40% said more info would affect their choice of restaurant or retail store
- BUT WHAT DOES THAT TELL US?

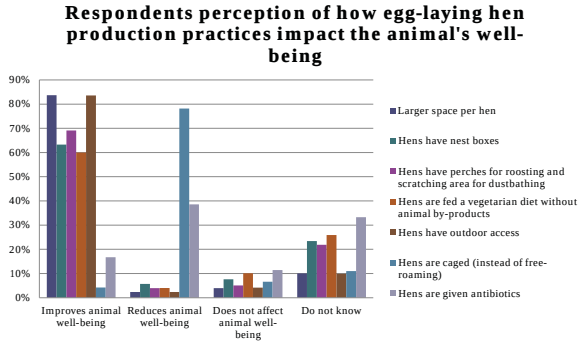
Food, Agricultural and Resource Economics

Consumers confused?

- Have remarkably little understanding
  - Ag production
  - Animal welfare
    - 80% say more space improve pig welfare
    - 65 % say gestation stalls improve pig welfare
  - They overestimate their level of understanding
- Don't know what they are getting now
  - 25% said don't know free range or free run eggs
  - 50% don't know on crate free pork
  - 47% don't know on antibiotic free pork

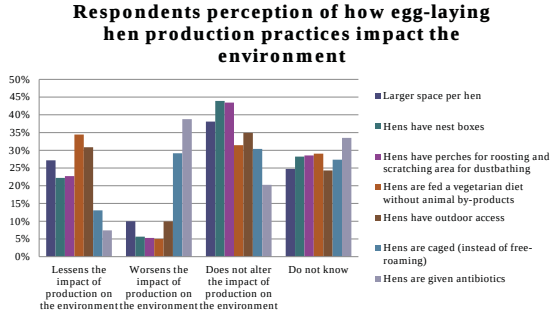
Food, Agricultural and Resource Economics

Perceptions of antibiotics



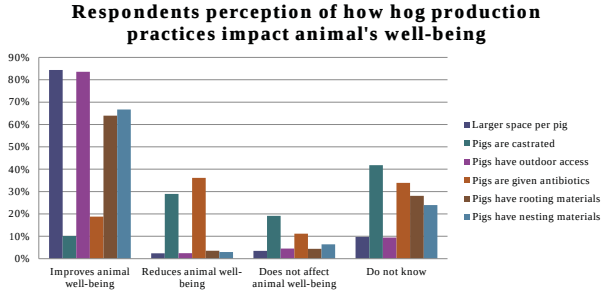
Food, Agricultural and Resource Economics

Perceptions of antibiotics



Food, Agricultural and Resource Economics

Perceptions of antibiotics



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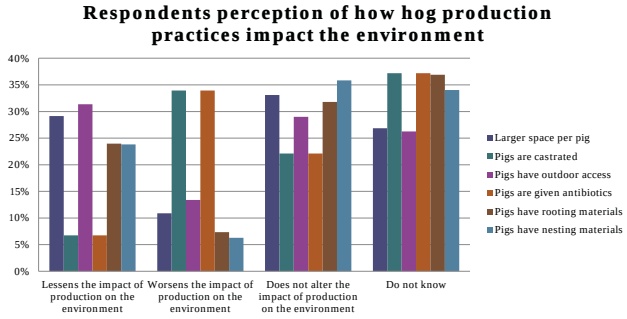
Perceptions of antibiotics



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Are antibiotics a concern?

- Many WTP studies
  - Varying degrees of premium suggested
  - Attribute priming
- Growth of “antibiotic free” and reduced use
  - Difficult to quantify
  - May also be hard to attribute just to that characteristic

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Are antibiotics a concern?

- Also studies about “clear conscience” versus willingness to pay.
  - Decrease in consumption of some products
  - Pork and beef most likely to decrease
  - Behaviour different in retail and food service

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Are antibiotics a concern?

- Heard the issue of trust this morning
- This also affects the brand.
  - Products
  - Stakeholders

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Consumer's Choices

- In 2013 animal welfare ranked in low in choice of restaurant
  - 2.2/100 . . . BUT
  - Much higher for a segment of the market – which has higher proportion of young people
  - Higher than "commitment to fair treatment of producers"

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Consumer's Choices

- In 2015 animal welfare ranked much higher in choice of restaurant
  - 8/100 . . .
    - Almost 4 fold increase in 2 years

So we need to look a bit deeper at what is happening

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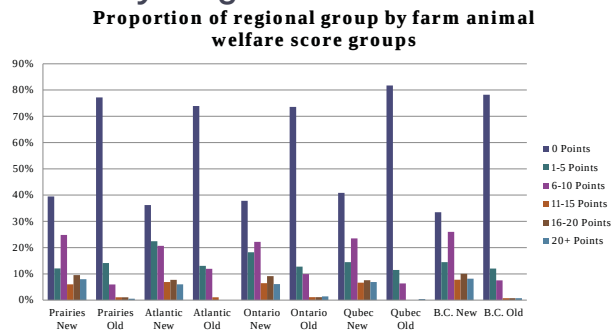
Scores by Region



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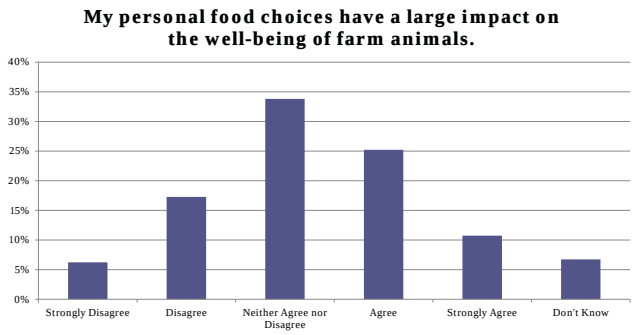
I can make a difference



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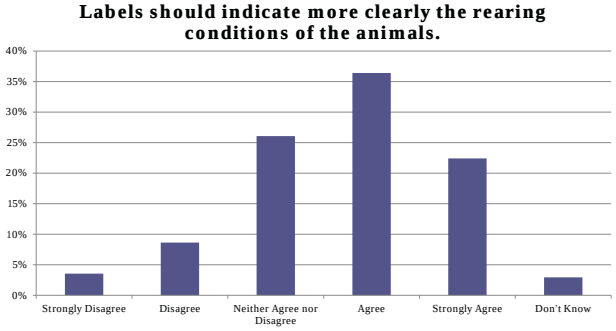
They want more information



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The rest of them

- Real danger in assuming they don't care about animal production
  - They may think things are relatively good now
  - That means uncertainty remains

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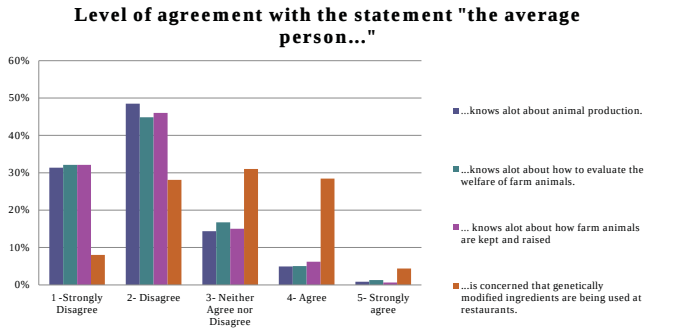
Very Little Understanding



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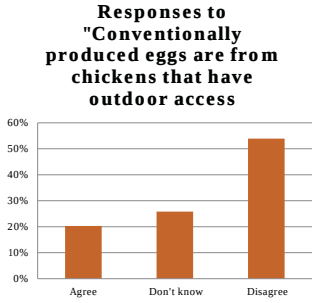
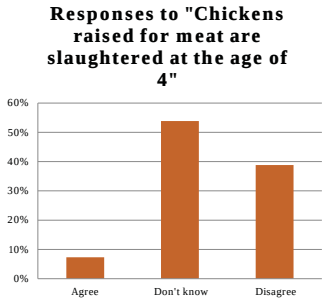
Consumers have very little understanding of production but expanding





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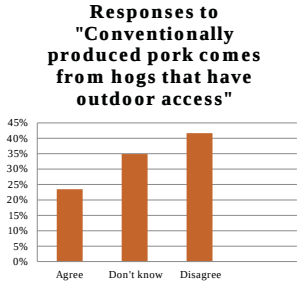
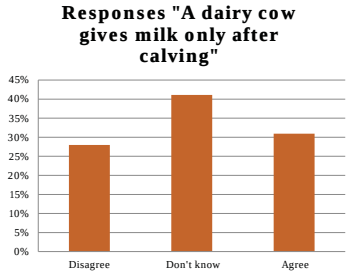
Consumers have very little understanding of production





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Consumers

- There may be some potential for nuance here
  - Understand that antibiotic use cannot be totally avoided
  - In-feed – "unnatural" or inappropriate

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Consumers

- Indication of willingness to pay for some sort of "reduced antibiotic" attribute
  - Some risk here
  - Evidence that it is affecting patronage decisions
  - Issue of "attribute priming."

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Antibiotics are different

- Striking a balance – tradeoffs
  - Not just cost
  - Welfare and resistance
  - Welfare and perception of risk
- Particularly a challenge given poor understanding by many stakeholders

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Some momentum

- The industry is doing things anyway
  - Consumers likely not aware
- Commitments bring awareness and demand
  - Attribute priming
- We all play a role in shaping the discussion

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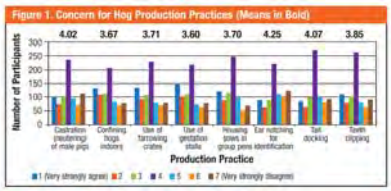
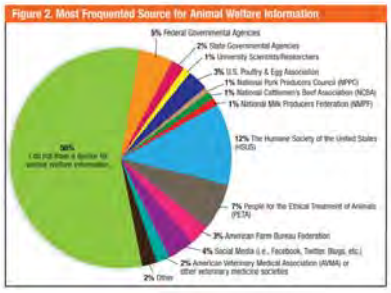
Consumers?

- McKendree et al, Purdue University





Thank You!

- mvonmass@uoguelph.ca
- @mikevonmassow

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Thank you

Graduate Student Posters

Affiches des étudiant(e)s diplômés

Decrease of Minimal Inhibitory Concentration of Essential Oils and Plant Extract in Presence of Complex Microbiota

Mélodie Langlais[1], Alexandre Thibodeau[1], Ann Letellier[1], Kathleen Sary[2], Philippe Fravalo[1]
[1] Chaire de recherche en salubrité des viandes, Pathology and Microbiology, University of Montreal, Saint-Hyacinthe, Canada
[2]Jefo, Saint-Hyacinthe, Canada

Abstract

Bacterial resistance to multiple antibiotics is a worldwide health problem. Various essential oils (EOs) and plant extracts have shown antibacterial properties. Therefore, they could be used in the development of novel feed additives in order to increase control of pathogens at the farm. However, efficiency of essential oils assessed in vitro failed to be confirmed when tested in vivo, as well in experimental or in field conditions. The aim of this study was to determine the in vitro antimicrobial activities of EOs (camphor, carvacrol, cinnamaldehyde, eugenol, peppermint) and plant extract (garlic) on food-borne pathogens (L. monocytogenes, S. Enteritidis) in presence or not of complex microbiota. The broth minimum inhibitory concentration (MIC) determination standard method was used, followed by an adapt method where MICs will be retested with the incorporation of complex microbiota mimicking digestive contents. Most of the EOs showed strong antimicrobial activities with MICs ≥ 1/1000 or equal to 1/10000. Interestingly, the results from the standard method were not confirmed when competitive flora were incorporate. The first results suggest an increase of MICs values by a factor 10 for most of essential oils tested in these new conditions, definitive results will be available for presentation. This study propose that in vivo inefficiency is probably related to competition flora and that incorporation of complex microbiota in MICs determination is essential for the first characterization of potential novel feed additives. Further studies will include other food-borne pathogens and non-pathogenic bacteria and the determination of the effect of EO on the total bacterial communities with 16S RNAr sequencing.

Feed Strategy to Improve the Microbial Quality of Meat

Stratégie alimentaire pour améliorer la qualité microbiologique de la viande

A.P. Kone[1,2], D. Cinq-Mars[1], F. Guay[1] et L. Saucier[1,2]

[1] Département des sciences animales, [2]Institut sur la nutrition et les aliments fonctionnels, Université Laval, Québec (QC), Canada, G1V 0A6

Abstract

Microbiological quality of our meat supply remains a concern as well as consumers' demand for minimally processed foods. Probiotics have been widely studied for their beneficial health effects, but little is known about their impact on carcass hygiene. Our hypothesis is that supplementing animal feed with a positive microflora can improve the microbial quality of meat products. A total of 144 female weaned Grimaud rabbits were divided into two experimental groups: 1) commercial diet (control) and 2) the same diet supplemented with Micocin® (Griffith Foods, Toronto, Canada), a commercial protective culture of *Carnobacterium maltaromaticum* CB1 (8-log CFU (Colony Forming Unit)/kg of feed). This culture produces three bacteriocins (carnocyclin A, piscicolin 126, carnobacteriocin BM1) able to inhibit *Listeria monocytogenes.* Each group was housed in a separate but similar room under strict biosecurity measures in order to control cross contamination between the two groups. Three specific genes (13S-cpg, ISR and CclA) were used to demonstrate the prevalence of *C. maltaromaticum* in meat from rabbits fed the diet containing Micocin®. The ground meat from both experimental groups was experimentally inoculated with a cocktail of five strains of *L. monocytogenes* (4-log CFU/g) and stored at 4 or 10 °C under aerobic and anaerobic conditions (0, 3, 6, 9, 12, 15 days). None inoculated meat served as a control. The most effective reduction of *L. monocytogenes* growth was observed with meat from the experimental group with Micocin® stored under anaerobic conditions at 4 °C and reached 2.1 log after 15 days (P = 0.02). Future work should include meat from other species, the search for other organisms that can act as protective cultures, and the effect of their long-term use on meat and livestock farming environment.

Keywords: *Carnobacterium maltaromaticum* CB1, Micocin®, meat, *Listeria monocytogenes*.

Résumé

La qualité microbiologique des produits de viande demeure une préoccupation et la demande des consommateurs pour des aliments peu transformés se poursuit. Les probiotiques ont été largement étudiés pour leurs effets bénéfiques sur la santé, mais leur impact sur l'hygiène des carcasses reste à évaluer. Notre hypothèse consiste à valider que l'enrichissement de l'alimentation du bétail par une microflore positive peut améliorer la qualité microbiologique des produits finis. Un total de 144 lapines Grimaud sevrées ont été divisées en deux groupes expérimentaux : 1) diète commerciale (témoin) et 2) la même diète supplémentée avec Micocin® (Griffith Foods, Toronto, Canada), une culture protectrice commerciale de *Carnobacterium maltaromaticum* CB1 (8-log

UFC (unité formant une colonie)/kg de moulée). Cette culture produit trois bactériocines (carnocycline A, piscicoline 126, carnobactériocine BM1) capables d'inhiber *Listeria monocytogenes*. Chaque groupe a été logé dans une salle séparée sous des mesures strictes de biosécurité afin de contrôler la contamination croisée entre les groupes. Trois gènes spécifiques (13S-cpg, ISR et CclA) ont permis de démontrer la prévalence de *C. maltaromaticum* dans la viande provenant des lapins nourris avec la ration contenant Micocin®. La viande hachée provenant des deux groupes expérimentaux a été inoculée expérimentalement avec un cocktail de cinq souches de *L. monocytogenes* (4-log UFC/g) et entreposée à 4 ou 10 °C dans des conditions aérobes et anaérobes (0, 3, 6, 9, 12, 15 jours). La viande non inoculée a servi de témoin. La réduction de la croissance de *L. monocytogenes* la plus importante a été observée avec la viande provenant du groupe expérimental avec Micocin® entreposée en conditions anaérobes à 4 °C pour atteindre 2,1-log après 15 jours (P = 0,02). Les travaux futurs devront inclure la viande provenant d'autres espèces, la recherche d'autres organismes pouvant agir comme culture protectrice et l'effet de leur utilisation à long terme sur la viande et l'environnement d'élevage.

Mots clés : *Carnobacterium maltaromaticum* CB1, Micocin®, viande, *Listeria monocytogenes*.

Amino Acids Requirements and Utilization Differs between Feeding Programs

Aline Remus[1,2,3], Luciano Hauschild[2], Marie-Pierre Létournou-Montminy[3], Candido Pomar[1,2,3]

[1]Agriculture and Agri-Food Canada, Sherbrooke, Quebec, Canada, [2]Department of Animal Science, Univ. of São Paulo State, Jaboticabal, São Paulo, Brazil [3]Departement des sciences animales, Université Laval, Quebéc, Quebéc, Canada

Abstract

There is a large variation in nutrient requirements among pigs and therefore, feeding pigs individually with daily tailored diets, or in groups with a single feed, may require different levels of nutrients. The response to different levels of threonine (Thr): lysine (Lys) ratio (70, 85, 100, 115 and 130% of the 0.65 Thr: Lys ideal ratio) was studied in growing pigs individually fed using precision feeding (PF) techniques or raised in the conventional group feeding (GF) systems. A 21-day trial was performed in a 2×5 factorial design with 110 pigs (25 kg BW ± 0.80, 11 pigs per treatment) housed in the same pen and fed with computerized feeding stations. The total lean content was estimated by dual X-ray absorptiometry at day 0 and day 21 of the trial. Five pigs per treatment were slaughtered. Threonine intake increased linearly ($P < 0.05$) with increasing dietary Thr levels for PF (6.28 to 11.76 g/d) and GF pigs (6.85 to 11.01 g/d). The ideal Thr: Lys ratio for GF was 65 and maximized protein deposition in 150 g/d, while maximal protein deposition was not reached in PF pigs (126 to 159 g/d). Meaning that AA ratios used to feed groups cannot be used for PF. Dietary Thr supply increased Thr concentration in a quadratic manner in PF pigs without effect on GF pigs ($P < 0.05$). Threonine restriction can, therefore, modify longissimus dorsi AA composition based on the AA intake. Threonine concentration in the liver tended ($P < 0.10$) to be greater in PF than GF pigs (4.44 vs. 4.39 g), showing that AA retention in organs may be more efficient in PF than GF pigs and, under AA restriction, organs seem to be prioritized over muscles. Altogether these results indicate that feeding systems can affect the way pigs use Thr and, the Thr: Lys ratio that maximizes growing pig responses differ between conventional and precision feeding systems.

Keywords: ideal protein profile, precision feeding

Attenuating the Negative Effects of Eimeria Infection in Broiler Chickens with Yeast Nucleotide Supplementation

Leung, H.[1], Neijat, M[1]., R. Snyder[2], R. Patterson[3], J. Barta[2], N. Karrow1 and E. Kiarie[1]
[1]Department of Animal Biosciences, University of Guelph, Guelph, ON
[2]Department of Pathobiology, University of Guelph, Guelph, ON
[3]Canadian Bio-Systems Inc., Calgary, AL

Abstract

Nucleotides are involved in many biological processes at the cellular level and may have the potential to attenuate or remedy structural and functional gut damage caused by coccidiosis. The effects of yeast nucleotides (**YN**) on growth performance and gastrointestinal variables in broiler chickens challenged with *Eimeria* were investigated. A total of 360 d old male chicks (Ross 708) were placed in 24 floor pens and allotted to a corn-soybean meal diet without or with YN product (500 g/ton) and fed *ad libitum* for 17 d. On d10, birds in 6 pens/diet were orally given 1 mL of *Eimeria* culture (*E. acervulina* and *E. maxima*) and the rest given 1 mL of distilled water as control. Growth performance was assessed pre-and post-challenge and oocyst count from d 14 to 17. On d 15, 5 birds/pen were sacrificed for intestinal lesion scores and histomorphology. Diets had no effect ($P > 0.05$) on growth performance pre-challenge. Interaction ($P<0.01$) between YN and *Eimeria* was observed for jejunal villi height (**VH**) such that challenged birds fed YN had higher (533 *vs.* 447 μm) VH than non-YN birds. Challenged birds had depressed ($P<0.05$) weight gain relative to non-challenged birds and exhibited intestinal lesion scores and oocyte shedding. Birds fed YN had higher weight gain (178 *vs.* 158 g, $P=0.05$) and a trend for lower FCR (1.09 *vs.* 1.29, $P=0.078$) compared to non-YN birds. Supplemental YN had no effect on intestinal lesion scores and oocyte shedding. In conclusion, *Eimeria* challenge resulted in decreased growth and damaged intestinal structure; supplemental YN improved intestinal histomorphology and growth. Supplementation of YN may offset the negative effects of *Eimeria* through improvement of intestinal absorptive surface to support growth.

Keywords: Broiler, yeast nucleotides, *Eimeria*, growth performance, intestinal lesions, histomorphology

Chemical Composition and Available Energy Contents of Canola Meal from Canadian Crushing Plants

Maral Rahmani[1], Anna Rogiewicz[2], and Bogdan A. Slominski[3]

[1]M.Sc. student of Poultry Nutrition, Rahmanim@myumanitoba.ca
[2]Adjunct Professor of Poultry Nutrition, Anna.Rogiewicz@umanitoba.ca
[3]Professor of Nutritional Biochemistry, Bogdan.Slominski@umanitoba.ca

Department of Animal Science, University of Manitoba, Winnipeg, Canada R3T 2N2

Abstract

Samples of canola meal (CM) were collected from thirteen canola crushing plants in Canada to determine the chemical composition, apparent metabolizable energy (AME_n) content, and fat digestibility in broiler chickens. On average, the contents of various components in % DM were as follows: CP (Nx6.25) 40.3 (from 38.9. to 43.1); ether extract (EE) 2.9 (from 2.4 to 3.8); acid hydrolyzed EE (AEE) 6.0 (from 3.6 to 7.2); sucrose 5.9 (from 5.6 to 6.2); oligosaccharides 3.2 (from 2.9 to 3.5); total P 1.13 (from 1.05 to 1.20); non-phytate P 0.33 (from 0.25 to 0.40); NDF 30.7 (from 28.3 to 34.0); total dietary fiber 40.0 (from 37.4 to 43.3); NSP 23.3 (from 22.4 to 24.2); neutral detergent insoluble CP (NDICP) 5.0 (from 3.4 to 7.5); lignin 11.7 (from 10.2 to 13.9) and in µmol/g DM, glucosinolates 2.68 (from 1.05 to 5.18). The mean AA contents were: lysine 2.19 (from 2.03 to 2.33); arginine 2.28 (2.14 to 2.39); methionine 0.74 (0.68 to 0.79); cystine 0.92 (0.83 to 1.01); threonine 1.57 (1.47 to 1.63). Among AA, lysine in particular was significantly different ($P<0.05$) in CM from different crushing plants. The AME_n content of CM samples averaged 1,789 kcal/kg DM and ranged from 1,566 to 2,064 kcal/kg DM. Digestibilities of EE and AEE (total fat) averaged 81 and 53%, respectively, which accounted for 2.1 and 3.1% DM of digestible EE and AEE contents, respectively. The difference representing the contents of soapstocks and gums accounted for only 0.9% DM. In summary, variations in the chemical composition of CM would primarily be associated with differences in the extent of heat treatment used in different crushing plants. Low digestibility of AEE would indicate that soapstocks and gums are poorly digested and contribute less energy to the AME_n content of CM than EE.

Keywords: canola meal, chemical composition, AME_n, fat digestibility

Deoxynivalenol's Impact on the Vaccine Response to PRRS and Circovirus-2

Impact du déoxynivalénol sur la réponse vaccinale du SRRP et du Circovirus-2

Kristina Dumont[1], Frédéric Guay

[1] Étudiante à la maîtrise en sciences animales/Département des sciences animales, Université Laval, Québec, QC, kristina.dumont.1@ulaval.ca

Abstract

Mycotoxins, such as deoxynivalenol (DON), are known to affect the growth performance of pigs, primarily by reducing feed intake. It is also recognized that DON can alter the immune functions, including the vaccine response, as well as the oxidative status of animals. The purpose of this study was therefore to determine the effects of DON-contaminated feeds and antioxidant supplements or antimycotoxin additives on the vaccine response of pigs to the porcine reproductive and respiratory syndrome (VSRRP) virus and Circovirus-2. Three hundred and thirty-two piglets were fed one of the three contaminated diets of 0.70, 1.5, 2.5 mg/kg DON and three other rations containing 2.5 mg/kg DON plus antioxidant supplements (Vitamins A and E 20,000 IU/kg and 200 IU/kg, organic selenium (yeast enriched) instead of sodium selenite, grape seed extract) or antimycotoxin additive (aluminosilicate hydrate of sodium and calcium) or a combination of the two supplements over a 35-day period. The piglets were all vaccinated on day 7 with the VSRRP and Circovirus-2 vaccines. The weight of the piglets was measured and blood samples were taken at day 35. No significant DON effects on daily feed intake, feed efficiency and daily average gain of piglets were observed. However, the body weight at day 35 decreased linearly with the DON contamination. The combination of supplements tended to increase the body weight at day 35. The DON contamination increased lymphocyte proliferation in vitro after concavalin A stimulation with a maximum value at 1.5 mg/kg DON. In vitro proliferation of lymphocytes in the presence of Circovirus-2 increased linearly in pigs exposed to DON. Pigs fed DON contaminated diet also had a higher IgG concentration with a maximum value at 1.5 mg/kg DON. The combination of supplements significantly increased the IgG concentration against VSRRP compared to 2.5 mg/kg DON. This study showed that a DON contamination at 1.5 mg/kg stimulated lymphocyte proliferation and the vaccine response to VSRRP and that the combination of antioxidant supplements and antimycotoxin additive can also positively influence this vaccine response to VSRRP.

Résumé

Les mycotoxines, comme la deoxynivalénol (DON), sont connues pour affecter les performances de croissance des porcs, principalement par une réduction de la prise alimentaire. Il est également reconnu que DON peut modifier la réponse immunitaire, incluant la réponse vaccinale, ainsi que le statut oxydatif des animaux exposés. La présente étude visait donc à déterminer les effets d'aliments contaminés par le DON et de suppléments en antioxydants ou en un additif

antimycotoxine sur la réponse vaccinale des porcs contre le virus du syndrome reproducteur et respiratoire porcin (VSRRP) et le Circovirus-2. Trois cent trente-deux porcelets ont été alimentés avec l'une des trois rations contaminées de 0,70, 1,5 2,50 mg/kg de DON ainsi que trois autres rations contenant 2,5 mg/kg de DON plus un supplément antimycotoxinique (aluminosilicate hydraté de sodium et de calcium), ou un supplément d'antioxydants ((vitamines A et E (20 000 UI/kg et 200 UI/kg, sélénium organique en lien et place du sélénite de sodium (levures enrichies en sélénium)) ou encore une combinaison des deux suppléments pendant une période de 35 jours. Les porcelets furent tous vaccinés au jour 7 avec les vaccins contre le VSRRP et le Circovirus-2. Le poids des porcelets a été mesuré et des échantillons sanguins ont été prélevés au jour 35. Aucun effet significatif de DON sur la prise alimentaire quotidienne, l'efficacité alimentaire et le gain moyen quotidien des porcelets ne fut observé. Toutefois, le poids au jour 35 tendait à diminuer linéairement avec l'augmentation du DON; l'ajout de la combinaison des suppléments tendait à augmenter le poids à jour 35. La contamination au DON a augmenté la prolifération lymphocytaire *in vitro* après stimulation par Concavalin A avec une valeur maximale à 1,5 mg/kg de DON. La prolifération *in vitro* des lymphocytes en présence de Circovirus-2 a augmenté linéairement chez les porcs exposés au DON. Les porcs exposés au DON ont aussi eu une concentration d'IgG plus élevée avec une valeur maximale à 1,5 mg/kg de DON. De plus, l'ajout de la combinaison des suppléments a significativement augmenté la concentration en IgG contre le VSRRP comparativement au traitement 2,5 mg/kg de DON. Cette étude a montré qu'une concentration en DON de 1,5 stimule la prolifération lymphocytaire et la réponse vaccinale contre le VSRRP et que la combinaison des suppléments en antioxydants et antimycotoxine peuvent aussi agir positivement sur cette réponse vaccinale au VSRRP.

Mots clés: déoxynivalénol, porcs, antimycotoxine, antioxydant, VSRRP, Circovirus-2, réponse vaccinale

Effects of Dietary *Fusarium* Mycotoxins and Matched Feeding on Broiler Performance, Feeding Behavior and Duodenum Morphology

Anhao Wang[1], Karen Schwean-Lardner[1], Natacha Hogan[1,2]

[1] Department of Animal and Poultry Science, [2] Toxicology Centre, University of Saskatchewan, Saskatoon, SK, Canada, S7N 5A8

Abstract

A 28d matched feeding trial was conducted to separate the impacts of *Fusarium* mycotoxins (primarily deoxynivalenol, DON) on broiler chicken feed intake from effects on performance, feeding behaviors and gut morphology. Ross 308 eggs were randomly divided into two equal groups and set in incubators 24h apart. At hatch, 72 healthy males were randomly selected and assigned to two dietary treatments (control vs. mycotoxin diet) with 6 birds/cage and 6 cages/ diet. Diets were naturally-contaminated with DON at 3 ppm in starter (1-21d) and 2.4 ppm in grower (22-28d) or < 0.1 ppm in control diets. The first set of birds were *ad libitum* fed and feed intake was measured daily. The second set of birds was subjected to matched feeding, where amount of diet provided to each matched-fed cage was calculated as: number of birds in a cage * average feed intake of *ad libitum* mycotoxin-fed birds at the same age. Three hours after feed was provided, feeding behavior was video recorded for one hour daily. Growth performance (body weight, average daily gain, feed to gain ratio) was measured on 7, 14, 21 and 28d post-hatch. At 28d, six birds per treatment were randomly sacrificed for histopathological assessment of duodenum morphology. As expected, matched-fed birds were lighter than *ad libitum* fed birds through experiment ($P<0.05$). Within matched-fed birds, dietary treatments did not affect performance ($P>0.05$). During the starter phase, dietary treatments did not affect growth performance ($P>0.05$). During 22-28d, control birds consumed more feed (148.6 vs. 138.4 g/d, $p<0.05$) and gained more weight (94.5 vs. 90.2 g/d, $p<0.05$) than mycotoxin-fed birds. Mycotoxin-fed birds spent more time at feeder (16.7 % vs. 11.7 %, $p<0.05$) compared to the control birds. Time at the feeder was not affected by *ad lib* or matched feeding during the same period ($P>0.05$). Duodenum morphology was not affected by treatments ($p>0.05$). Taken together, *Fusarium* mycotoxins suppressed broiler growth performance by inducing taste aversion and partial feed refusal.

Effect of Dietary Supplementation of Plant-Based Products on Oxidative Status of Weanling Piglets

Sirini. B. Amarakoon[1,3], Balachandar Jayaraman[1], Yaw. L. Siow[2,3,4], Suvira Prashar [2,3], Yvette Shang[1,3], Karmin O[1,3,4] and Martin Nyachoti[1,]*
[1]Department of Animal Science, University of Manitoba, Winnipeg, MB, Canada, R3T 2N2, [2]Agriculture and Agri-Food Canada, [3]St. Boniface Hospital Research Centre, Winnipeg, Canada, MB R2H 2A6,
[4]Department of Physiology and Pathophysiology, University of Manitoba Winnipeg, MB, Canada, R3T 2N2,[*]martin.nyachoti@umanitoba.ca

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Abstract

Oxidative stress is highly detrimental to swine health. *E. coli* infection in weanling piglets is a huge challenge worldwide which causes diarrhea related death of piglets. Antibiotics have been included in weaner diets as an immune modifier and a growth promoter to tackle the situation. Recent concerns for antibiotic resistance have raised the need of finding alternatives for antimicrobials. The present study was carried out to evaluate the effect of dietary supplementation of barks and leaves mixture of a Canadian plant, Red osier dogwoods on oxidative status of *E. coli* challenged weanling piglets. Twenty eight weaned piglets were assigned to four diets consisting of a corn-wheat and soybean meal based diet (negative control, **NC**), NC plus 2% dogwoods (**ROD2**), NC plus 4% dogwoods (**ROD4)**, and NC plus antibiotics (**PC**). These piglets were orally challenged with enterotoxigenic *E. coli* (ETEC) K88+. Another group of 4 piglets fed the NC diet was kept in a separate room and served as an unchallenged control (**UC**). Oxidative stress biomarker malondealdehide (MDA) levels in the ileum and serum of the NC and ROD2 fed piglets were elevated ($P < 0.05$) and the antioxidant enzyme superoxide dismutase (SOD) activity levels in the ileum and serum of these two groups were decreased ($P < 0.05$) compared to other groups whereas ROD4 and PC groups showed MDA levels and SOD activity levels similar to that of UC. These results suggest that dietary supplementation of this plant material can attenuate oxidative stress in *E. coli* challenged weaned piglets similar to antibiotics thus showing a potential as an alternative to in-feed antimicrobials.

Keywords: antioxidants, *Escherichia coli,* oxidative stress, piglets, Red osier dogwoods

Feed Additives can Reduce the Impact of Sanitary Challenge by Bacterias: a Meta-Analytic Approach

Aline Remus[1,2,3], Luciano Hauschild[2], Marie-Pierre Létournou-Montminy[3], Candido Pomar[1,2,3]

[1]Agriculture and Agri-Food Canada, Sherbrooke, Quebec, Canada, [2]Department of Animal Science, Univ. of São Paulo State, Jaboticabal, São Paulo, Brazil [3]Departement des sciences animales, Université Laval, Quebéc, Quebéc, Canada

Abstract

Health challenges can affect production costs because they impair broiler performance. This meta-analysis was conducted to evaluate the performance responses of broiler chickens treated with feed additives (FA; prebiotic or probiotic or symbiotic), challenged by enteric bacteria. The database was composed of 65 articles, published between 1997 and 2012, with a total of 86,300 broilers, where 10,851 chickens were challenged with Clostridium spp., 4,890 chickens challenged with E. coli, and 45,703 chickens challenged with Salmonella spp., and the control group composed of 24,856 not challenged birds. The initial age was 10 d (1–18 d); final age: 21 d (12–44 d), dietary ME 2,961 kcal/kg (2,900–3,604 kcal/kg), CP: 21.77% (15.2–27.33%), total lysine: 1.34% (0.88–2.04%), total methionine: 0.72% (0.39–1.62). The meta-analysis involved three sequential analyses: graphical, correlation, and variance-covariance analysis. Growth rate of births challenged with bacteria but treated with FA was 2.9 % lower than in control births while it was 21.6 % lower in untreated births ($P<0.05$). The use of FA can reduce the impact of the sanitary challenge on birds' growth. In the regression equation for ADG as a function of ADFI showed that challenged and non-treated broilers had the maintenance affected (slope -3.5) by the sanitary challenge. Birds that were challenged and fed FA did not increase their maintenance requirements (slope 0.74); however, the curvilinear response showed that feed intake reduction worsened feed efficiency. Overall, FA can significantly reduce losses in ADG and ADFI in broilers challenged with enteric bacteria.

Keywords: probiotics, prebiotics, broilers

Gastrointestinal Physiological Responses to Feeding Fiber in Two Strains of Laying Hens

Alisha Wornath-Van Humbeck[1], Mohamed Neijat[1], Elijah Kiarie[1]
[1]Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada.

Abstract

The effects of feeding laying hens fibrous feed on the gastrointestinal physiology is not well characterized. Moreover, the differences between modern and heritage layer strains are not known. We investigated gastrointestinal responses in 57-wk Lohmann Select Leghorn-Lites (LSL) and Heritage White Leghorns (SHWL) fed ground (GOT) or unground (UGOT) oat hulls. The diets consisted of a control or a mixture of control with GOT or UGOT at a ratio of 80:20 wt/wt. A total of 288 hens (6 hens/cage, 24 cages for LSL and SHWL, respectively) were fed a control diet for 1 wk to collect production data as the basis for diet allocation. One hen per cage was sacrificed for baseline organ weights and diets were allocated within breed. Birds had free access to feed and were sacrificed on d 28. Organ weight change index (WCI) was calculated as a ratio of d 28 to baseline organ weights. The gizzard was the only baseline organ that differed between breeds, with SHWL showing 34% heavier gizzard than LSL ($P < 0.01$; 12.3 *vs.* 8.1 g/kg live BW). On d 28, there was no interaction ($P > 0.05$) between breed and diet on organ weight. LSL hens had heavier proventriculus than SHWL ($P < 0.1$; 3.7 *vs.* 3.3 g/kg live BW), which in turn had greater gizzard weights than LSL ($P < 0.01$; 17.0 *vs.* 13.4 g/kg live BW). There was breed and diet interaction ($P < 0.01$) on gizzard WCI such that LSL hens receiving oat hulls had the greatest gizzard WCI compared to control or SHWL hens fed any diet. Specifically, gizzard WCI for the control, GOT and UGOT diets were 1.01, 1.83 and 2.10, respectively for LSL and 0.91, 1.55 and 1.66 for SHWL. The LSL hens also had higher WCI for proventriculus ($P < 0.01$), small intestine ($P = 0.03$) and a trend ($P = 0.08$) for heavier liver than SHWL hens. In conclusions, the data showed the modern layer has the ability to adjust gut physiology in response to fibrous feed more than their predecessors.

Keywords: Laying hens, Gizzard, Fiber

Optimization of Starting Methods for Antibiotic-Free Poults

Optimisation des méthodes de démarrages chez le dindonneau sans antibiotiques

Violette Caron-Simard, Martine Boulianne, Maude Rhéaume

Chaire en recherche avicole, Faculté de médecine vétérinaire, Université de Montréal, 3200 Sicotte, St-Hyacinthe, QC, J2S 2M2, Canada

Abstract

Background. The starter period in turkey production has become more challenging since Type I antibiotics (very important in human health) were withdrawn in 2014 in the hatchery, with more mortalities at an early age. Starter methods in poults vary greatly in Quebec. In order to identify management practices for optimal poult start-up, 25 flocks across the province were observed and compared for performances.

Results and analysis. Total mortality rates at 10 days of age were lower in poultry houses where cloacal temperature ranges were the lowest, with a correlation of 0.55 (p=0.008). A 0.47 positive correlation was noted between mortality at 10 days of age and total mortality (p=0.02). Number of 1-day old poults with feed in their crop was highly correlated (0.64) with luminous intensity (p=0.02). At least 50 % of poults had consumed feed when luminosity at day 1 was higher than 25 lux in the house (p=0.0001).

Conclusions. Luminosity may encourage poults in looking for and consuming feed. Since poults need to access the feed as quickly as possible, producers should focus on this management parameter as well as on minimum cloacal temperatures of 103ºF and constant environmental temperatures inside the poultry house. Interestingly, recommended cloacal temperature in the poult is about 1.5ºF lower than in the chick. Improving conditions during the starter period and mortality rates at 10 days will lead to a reduction in total mortality, thus to an increased profitability for the producer.

Résumé

Contexte. La période de démarrage dans la production de dindons présente des difficultés accrues depuis le retrait en 2014 des antibiotiques de type I (très haute importance en médecine humaine) au couvoir avec plus de mortalités en bas âge. Les méthodes de démarrage de dindonneaux sont très variées au Québec. Afin d'identifier les pratiques de régie pour un démarrage optimal, vingt-cinq élevages de la province ont été observés et comparés selon leurs performances d'élevage.

Résultats et analyses. Les taux de mortalités totaux obtenus à 10 jours d'âge étaient inférieurs dans les poulaillers où les écarts de température cloacale étaient les plus faibles, avec une corrélation de 0,55 (p=0,008). Il y avait une corrélation positive de 0,47 entre la mortalité à 10 jours d'âge et la mortalité totale (p=0,02). Le nombre de dindonneaux à un jour d'âge présentant un jabot avec de la nourriture était fortement corrélé (0,64) à l'intensité lumineuse (p=0,02). Au moins 50% des dindonneaux avaient mangé lorsque la luminosité au jour 1 était supérieure à 25 lux dans le poulailler (p=0,0001).

Conclusions. La luminosité stimulerait donc les dindonneaux à trouver et consommer la nourriture. Comme les dindonneaux doivent accéder à la moulée le plus rapidement possible, les éleveurs devraient mettre l'accent sur ce paramètre de régie, ainsi que sur les températures cloacales minimales de 103°F et une uniformité de la température environnementale dans le poulailler. Il est intéressant de constater que la température cloacale recommandée chez le dindonneau est environ de 1.5°F plus faible que celle recommandée chez les poussins. L'amélioration des conditions de démarrage et des taux de mortalité à 10 jours se traduiront par une diminution de la mortalité totale, et donc une amélioration des profits pour l'éleveur.

Mots clés : régie, dindon, dindonneaux, température cloacale, luminosité, remplissage de jabot

Soybean Pelleting Temperature Affects Ileal Digestibility of Amino Acids in Broiler Chickens

John F Smillie[1]; Antoniel Franco[2]

[1] Ph.D. Student, Department of Animal and Poultry Science, University of Saskatchewan, SASKATOON, SK S7N 5A8, john.smillie@usask.ca; [2]Visiting Scholar, Department of Animal Production, Faculty of Veterinary and Animal Science, Universidade Federal do Parana, Curitiba, 80035-050, Brazil

Abstract

Hydrothermal treatment of feed products through pelleting typically results in improved animal performance. However, the focus of pellet production has been primarily focused on physical product quality and throughput not nutritional quality. The objective of this research was to determine the impact of pelleting conditions on the nutritional value of soybean meal (SBM) as well as pellet quality. Near Infra-Red Spectroscopy analysis was also used to predict changes to protein quality during pelleting. SBM was pelleted at five temperatures, 55, 65, 75, 85 and 95ºC. The ileal digestibility of amino acids in the pelleted meals as well as untreated SBM were determined in broiler chickens. The digestibility of lysine, threonine, valine, arginine and isoleucine responded in a quadratic manner to processing temperature with maximum digestibility being between 65-75ºC, however pellet durability improved linearly. An additional study also examined the interaction between amino acid digestibility and particle size. The results suggest pelleting between 65-75ºC is optimal for amino acid digestibility. The improvements during the initial temperature increase can be due to changes in the structure and solubility of the proteins and the detrimental effect seen at higher temperatures are likely due to Maillard reactions. Additional research is required to determine if other ingredients respond similarly, but it is expected other protein meals would be similar. In conclusion, modest pelleting temperatures (approximately 70ºC) are recommended as they promote maximum amino acid digestibility and reasonable pellet quality. Application of these findings will hopefully prevent the over processing of feed products and contribute to understanding of pelleting as a science.

Keywords: pelleting temperature, soyabean meal, amino acid digestibility, NIR.

Studying the Chicken Caecal Microbiota: Important Variation Observed between True Biological Replicates even in Controlled Conditions.

Alexandre Thibodeau[1] et Philippe Fravalo[1]
[1] Chaire de Recherche industrielle du CRSNG, Faculté de médecine vétérinaire, Université de Montréal, Saint-Hyacinthe, alexandre.thibodeau@live.ca

Abstract

Reduction of costs and increase of the instruments availability liberalized high-throughput sequencing as a method to characterize the animal microbiota. These recent years, a drastic rise in term of number of studies analyzing the animal microbiota can be observed, but very few of them are actually replicated. This study objective was to assess how much different the caecal microbiota of chickens, raised in level 2 bioconfinement facility, can be between independent replicate of the same experimental design. For this, MiSeq 16S ribosomal gene amplicon sequencing data (V4 region) of two different experimental settings, both replicated, were analyzed together and compared according to the experimental replicate. Raw sequences were processed and analyzed using Mothur, based on the recommended SOP adapted by our laboratory. The structure and composition of the chicken caecal microbiota were assessed using the OTU and phylotype approach. For both experimental settings and approaches, it could be observed on the 2D non-metric multidimensional scaling graphical representation of the microbiota structure that within an experimental setting, replicates formed distinct groups. This was statistically corroborated by the analysis of molecular variance (AMOVA). The replicates were also different in term of the observed microbiota composition. Indeed, a linear discriminant analysis effect size (LEfSe) was able to associate specific bacterial markers to the different replicates. Based on these results, it clearly appears that the chicken caecal microbiota is and can be statistically observed to be different between replicates, even when the birds are raised in the most similar conditions possible in a bioconfinement 2 animal facility. It therefore indicates that true independent replication is crucial when analyzing the chicken caecal microbiota in order to identify differences that are being observed for a given condition.

Assessing the Effect of Body Condition Loss on Hepatic and Ovarian Tissue Function During the Transition Period in Dairy Cows

Y Schuermann[*1], A St. Yves[1], N Dicks[1], V Higginson[1], R Bohrer[1], M Taibi[1], MP de Macedo[1], E Madogwe[1], A Mustafa[1], B Baurhoo[1,2], V Bordignon[1]and R Duggavathi[1]; McGill University, Montreal, Quebec, Canada[1], Bélisle Inc., Saint-Mathias-sur-Richelieu, Quebec, Canada[2]*

Abstract

The Canadian dairy industry has bred top genetic cows. Still, many of these cows fail to remain in the herd for more than two lactations. The transition period is a stressful adaptation time for dairy cows. The inability to meet her energy requirements leads to a state of negative energy balance, often accompanied by excessive lipid mobilization from adipose tissue. Body condition scoring (BCS) provides a visual appraisal of lipid mobilization. Our aim was to investigate the effect of body condition loss on hepatic and ovarian tissue function in dairy cows during the transition period until onset of breeding. Holstein cows were studied from 4 weeks pre-calving until 8 weeks post-calving and retrospectively grouped by changes in BCS. Group 1 (G1) (n=9) consisted of cows that lost < 0.75(moderate) BCS and Group 2 (G2) (n=8) cows that lost ≥ 0.75(severe) BCS during early lactation. We collected liver biopsies at -3 weeks, near day of calving and +7 weeks. The last liver biopsy was accompanied by follicular aspiration of the dominant follicle and retrieval of granulosa cells (GCs). We evaluated mRNA levels in hepatocytes and GCs. Reproductive competence was evaluated through mRNA abundance of genes required for follicular development (*FSHR* and *LRP8*) in GCs, but remained constant among groups. In hepatocytes, *CYP7A1* transcripts gradually increased (P<0.07) in G2 from -3 weeks to +7 weeks, but remained constant in G1. Other transcripts involved in lipid metabolism (*LDLR*, *ACAT1* and *SCARB1)* remained unaltered between groups. Cows facing elevated BCS loss experience a gradual increase in hepatic *CYP7A1,* an enzyme involved in bile acid synthesis from cholesterol, suggesting that G2 faced a greater need for cholesterol removal from the body. Thus, a deeper knowledge of changes in liver tissue can assist in developing feed additives to support the transition period.

Keywords: Ovary, Liver, BCS

Effect of Delaying Colostrum Feeding on Passive Transfer and Intestinal Bacterial Colonization in Neonatal Male Holstein Calves

Amanda J. Fischer, Yang Song, Zhixiong He, Leluo Guan, Michael A. Steele
Department of Agricultural, Food, and Nutritional Science, University of Alberta, Edmonton, AB, T6G 2P5, Canada, afischer@ualberta.ca

Abstract

Calves are born without an active immune system, and therefore rely on good-quality, adequate volumes of colostrum to ensure the passive transfer of IgG. Despite this knowledge, poor colostrum management still occurs on farm, with one of the main reasons for failure of passive transfer being due to feeding colostrum more than 6h after birth. The objective of this study was to investigate how delaying the first colostrum feeding can impact the passive transfer of IgG, as well as bacterial colonization in the intestine of neonatal dairy calves. Twenty-seven male Holstein calves were randomly assigned to 1 of 3 treatments at birth: calves were fed colostrum before 1h after birth (0h, n=9), at 6h after birth (6h, n=9), or at 12h after birth (12h, n=9). Calves were fed pooled colostrum at their respective feeding times at 7.5% of birth body weight, and fed milk replacer at 2.5% every 6h thereafter. Blood samples were taken every 3h, and at 51h of life calves were euthanized and tissue and digesta of the distal jejunum, ileum and colon were collected. Calves fed colostrum at 0h had significantly higher ($P<0.001$) serum IgG concentrations (g/L;24.77 ± 1.91) compared to 6h (17.13 ± 0.91) and 12h calves (16.88 ± 1.50). In addition, 0h calves had a higher prevalence ($P<0.10$) of *Bifidobacteria* (1.24 ± 0.64) and *Lactobacillus* (0.26 ± 0.08) attached to colon tissue compared to 12h calves (0.12 ± 0.02 and 0.07 ± 0.02, respectively). There were no differences ($P>0.10$) in *E. coli, Clostridium*, and *Faecalibacterium* intestinal colonization among treatments. These findings suggest that feeding dairy calves colostrum immediately after birth can increase the passive transfer of IgG and the colonization of beneficial bacteria in the colon; both of which are hypothesized to assist in protecting the calf from enteric infections during the pre-weaning period.

Keywords: passive transfer, dairy calf, bacterial colonization

The Effect of Nipple Bottle vs Esophageal Tube Feeding of Colostrum on Absorption of IgG and Plasma Glucagon-like Peptide-2 Concentrations

M. Desjardins-Morrissette[1], J. K. van Niekerk[1], D. Haines[2], T. Sugino[3], M. Oba[1], M. A. Steele[1]

[1]Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB T6G 2P5, Canada

[2]The Saskatoon Colostrum Co. Ltd., Saskatoon, Canada

[3]The Research Center for Animal Science, Graduate School of Biosphere Science, Hiroshima University, Higashi-Hiroshima, Japan 739-8528

Abstract

Colostrum management is essential for calf survival and vitality in the dairy industry, and current practices on farm are to feed colostrum through either an esophageal tube or a nipple bottle. The objective of this study was to determine if feeding colostrum to newborn calves through an esophageal tube, compared with a nipple bottle, affects passive transfer of IgG and plasma glucagon-like peptide-2 (GLP-2) concentrations. Twenty newborn Holstein bull calves (birth BW= 44.8 ± 4.13 kg; mean ± SD) were fed 3L of colostrum replacer (200 g IgG) through either an esophageal tube or nipple bottle at 2 h after birth. The following meals of pooled whole milk were fed at a volume of 3L at 12, 24, 36 and 48 h after birth. Maximum concentration (Cmax) of serum IgG was 24.2 and 24.7 ± 0.58 mg/mL, time to maximum concentration (Tmax) was 786 and 966 ± 161 min, and apparent efficiency of absorption of IgG (AEA) was 52.7 and 53.2 ± 1.63% for the bottle and tube treatments, respectively. There was no difference between treatments for serum IgG maximum concentration, Tmax, or AEA ($P > 0.44$). There was also no significant treatment effect on GLP-2 Cmax, Tmax, or area under the curve ($P > 0.20$). There was a significant time effect ($P < 0.001$), however, indicated by an increase in GLP-2 concentrations following colostrum feeding. These results indicated that feeding colostrum at a volume of 3L through either an esophageal tube or nipple bottle accomplishes adequate passive transfer of IgG and increases GLP-2 concentrations after colostrum feeding. Feeding colostrum through either an esophageal tube or nipple bottle is a viable method to ensure successful colostrum management on farm.

Keywords: colostrum, esophageal tube, nipple bottle

The Effects of Yeast-Derived Microbial Protein and Live Yeast on Metabolic Health and Lactational Performance of Transition Dairy Cows

V Higginson[1], B Baurhoo[1,2], Y Schuermann[1], A St- Yves[1], M Taibi[1] and A Mustafa[1]

[1] McGill University, Montreal, QC
[2]Bélisle Solution Nutrition Inc., St-Mathias sur Richelieu, QC

Abstract

In dairy cows, the transition period is burdened by high nutrient demands at a time when DMI is lowest. Despite much research on dietary management during the transition period, cow health during this time remains a large concern. Yeast-derived microbial protein (YMP) had no adverse effects on lactating cows but the effects on transition cows are unknown. Two studies were conducted to evaluate the effects of YMP supplementation alone or with live yeast on the performance and metabolic status of transition cows. In study 1, twenty-seven Holstein cows were blocked by calving date and parity and randomly assigned to receive either a control (0g YMP) or treated (50g YMP prepartum and 200g YMP postpartum) pellet. The study started 21 d prior to calving date and ended 28 d postpartum. Study 2 included twenty-six cows and followed the same protocol except that treatment included a control (no supplementation) or YMPL (100g YMP prepartum and 200g YMP postpartum with 10g live yeast) diet. In both studies, DMI and milk yields were recorded daily and milk samples were obtained twice weekly. Blood samples collected on d -21, -14, -7, -3, -1, 1, 3, 7, 14, 21 and 28 relative to calving and were analyzed for metabolic parameters. Study 1 showed no effect of treatment on DMI and milk performance. Interestingly, YMP supplementation reduced serum non-esterified fatty acids levels from d 3 postpartum until the end of the study and β-hydroxybutyrate levels were lower on d 3 and 7 postpartum. In YMP cows, serum glucose levels were higher on d 3 and 7 postpartum whereas aspartate transaminase levels were lower on d 14 and 21 postpartum. In study 2, there was no effect of YMPL on DMI or milk performance. Overall, YMP may improve metabolic health of transition cows by reducing adipose tissue mobilization without affecting cow performance. However, the addition of live yeast had no additional benefit on cow performance.

Keywords: Transition cow, Yeast, Metabolic health

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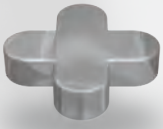

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