



2018 ANIMAL NUTRITION
CONFERENCE OF CANADA



2018 COLLOQUE DE NUTRITION
ANIMALE DU CANADA

Proceedings

Cahier
de conférences



2-3

May/mai 2018

Edmonton
Alberta

Cutting edge nutritional strategies for improving
performance, profitability and sustainability

Stratégies nutritionnelles de pointe pour améliorer
le rendement, la rentabilité et la durabilité

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Commanditaire, symposium pré-colloque

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Welcome from ANAC / Bienvenue de l'ANAC

The Animal Nutrition Association of Canada (ANAC) is honoured to organize and host the Animal Nutrition Conference of Canada (ANCC). This annual learning and networking opportunity serves as a bridge for cutting edge research to be shared with industry in the Canadian and international animal nutrition community. This national conference also affords an occasion to discuss new challenges, trends, and changes with colleagues and stakeholders across the country, which is especially important as the Canadian *Feeds Regulations* will be undergoing the most significant overhaul in over 30 years. The success of the feed industry is only as strong as the success of our customers. The topics addressed at ANCC ensure we have the best tools so that nutrition is an integral part of the success of our livestock sectors.

The conference organizing committee has planned an exceptional program and ANAC would like to thank them for their dedication. We would also like to thank our industry partners, as a conference of this caliber is not possible without your generosity and support. ANAC would also like to express our gratitude to the world-class speakers and the graduate students who will be sharing their knowledge and research over the next couple of days.

We hope you enjoy the second edition of the ANCC and can bring back innovative ideas and new partnerships to your daily work.

Melissa Dumont, agr.

Executive Director, Animal Nutrition Association of Canada

L'Association de nutrition animale du Canada (ANAC) a, encore une fois, l'honneur d'organiser et d'être l'hôte du Colloque de nutrition animale du Canada (CNAC). Ce rassemblement annuel, qui offre d'innombrables occasions d'apprentissage et de réseautage, sert de pont vers la recherche à la fine pointe qui sera partagée avec la communauté de l'industrie de nutrition animale au Canada et de par le monde. Ce colloque national offre aussi l'occasion de discuter des nouveaux défis, des tendances et des changements avec des collègues et intervenants de tout le pays, ce qui revêt un intérêt particulier à la lumière du remaniement le plus important en 30 ans du Règlement sur les aliments du bétail canadien. Le succès de l'industrie de l'alimentation du bétail dépend du succès de nos clients. Les sujets abordés au CNAC assureront que nous ayons en mains les meilleurs outils pour que la nutrition fasse partie intégrante du succès de nos secteurs d'élevage du bétail.

Le Comité organisateur du colloque a préparé un programme exceptionnel et l'ANAC remercie ses membres pour leur dévouement à la cause. Nous remercions également nos partenaires de l'industrie puisqu'un colloque de ce niveau ne peut avoir lieu sans leur générosité et leur appui. Enfin, nous voulons exprimer notre gratitude aux conférenciers de calibre mondial et aux étudiants diplômés qui partageront leurs connaissances avec nous au cours des quelques prochains jours.

Nous espérons que vous aimerez la deuxième édition du CNAC et que vous pourrez en appliquer les idées novatrices et les nouveaux partenariats à votre travail quotidien.

Melissa Dumont, agr.

Directrice exécutive, Association de nutrition animale du Canada

Organizing Committee 2018 / Comité organisateur 2018

We are honoured to welcome you to the 2nd edition of the Animal Nutrition Conference of Canada (ANCC), which is being held in the beautiful city of Edmonton. This year, the organizing committee, which consists of respected scientists and industry members from Eastern and Western Canada, have selected the theme “Cutting Edge Nutritional Strategies for Improving Performance, Profitability, and Sustainability”. The feed industry is at a crossroads, both from a regulatory and societal point of view. Our industry will need to adapt to changes in how antimicrobial compounds can be used in animal feeds as well as the range of challenges associated with an ever-increasing global demand for high quality affordable protein products.

The speakers at this year’s conference are experts in how the science of animal nutrition can be applied to industry and their talks should provide new insights into how their research and innovations can be used to overcome the daily production challenges faced by animal nutrition professionals. In organizing the program, our goal was to include ample opportunities for networking as well as showcase our student researchers and industry partners. We sincerely thank our conference sponsors and all participants for supporting this event. Enjoy the conference!

Nous sommes honorés de vous accueillir à la 2e édition du Colloque de nutrition animale du Canada (CNAC), qui se tient dans la belle ville d'Edmonton. Cette année, le Comité organisateur, composé de scientifiques respectés et de membres de l'industrie de l'Est et de l'Ouest du Canada, a choisi le thème « Stratégies nutritionnelles de pointe pour améliorer la performance, la rentabilité et la durabilité ». L'industrie de l'alimentation animale est à la croisée des chemins, tant du point de vue réglementaire que sociétal. Notre industrie devra s'adapter aux changements dans la façon dont les composés antimicrobiens peuvent être utilisés dans les aliments pour animaux, ainsi qu'à toute la gamme de défis associés à une demande mondiale sans cesse croissante de produits protéiques abordables et de haute qualité.

Les conférenciers invités au colloque de cette année sont des experts dans l'application de la science de la nutrition animale à l'industrie, et nous croyons que leurs exposés permettront de dégager de nouvelles idées sur la façon dont leurs recherches et innovations peuvent être utilisées pour surmonter les défis de production que doivent affronter les professionnels de la nutrition animale dans leur travail quotidien. En organisant le programme, nous nous sommes fixés l'objectif d'inclure de nombreuses occasions de réseautage et de mettre en évidence nos étudiants chercheurs et partenaires de l'industrie. Nous remercions sincèrement les commanditaires du colloque et tous les participants pour leur soutien à l'endroit de cet événement. Profitez du colloque!

Rob Patterson, Canadian Bio-Systems Inc.
(Program Chair/Président du programme)

Theunis Wessels, Poultry Partners Inc.
(Sponsorship Chair/Président des commandites)

Doug Korver, University of Alberta
(Academic Chair/Président de l'académie)

Karen Beauchemin, Agriculture & Agri-Food Canada
(Lethbridge)

Raylene Boehmer, Trouw Nutrition Canada

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

Mark Cameron, Nutrisource Inc.

Ralf Dietert, LV Lomas

Dan Ganesh, Bos Nutrition Services Inc.

Darryl Gibb, Gowans Feed Consulting

Paul Groenewegen, Masterfeeds, an Alltech
Company

Zahid Nasir, Trouw Nutrition

Masahito Oba, University of Alberta

Mohsen Pourabedin, Cargill Ltd.

Sharon Robinson, Halchemix Canada Inc.

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

Doug Teitge, DSM Nutritional Products Canada Inc.

2018 ANCC CONFERENCE PROGRAM

PROGRAMME DU COLLOQUE CNAC 2018

May 2nd to 3rd, 2018 – Chateau Lacombe Hotel, Edmonton, Alberta
 Les 2 et 3 mai 2018 – Hôtel Château Lacombe, Edmonton, Alberta

Cutting edge nutritional strategies for improving performance, profitability, and sustainability

Stratégies nutritionnelles de pointe pour améliorer le rendement, la rentabilité et la durabilité

TUESDAY, MAY 1ST – 4 TO 6 PM / MARDI LE 1^{ER} MAI – 16 H À 18 H

Welcome Cocktail / Cocktail de bienvenue (La Ronde Restaurant, Chateau Lacombe)

Join us on the 24th floor of the hotel's revolving restaurant and meet with industry associates for a spectacular 360° view of Edmonton. Registration packages will also be ready for pickup.

Rendez-vous au 24^e étage du restaurant tournant de l'hôtel pour rencontrer des associés de l'industrie et admirer une vue à 360° du magnifique panorama d'Edmonton. Vous pourrez aussi y prendre votre trousse d'inscription.

WEDNESDAY, MAY 2ND – MORNING / MERCREDI LE 2 MAI – MATIN

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

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

Pre-Conference Symposium <i>Symposium précolloque</i> Room/Salle: Salon A & B			HALCHEMIX research proven nutrition
A New Look at Specialized Nutritional Technologies <i>Un nouveau regard sur les stratégies nutritionnelles spécialisées</i>			
7 :00	Registration and hot breakfast / <i>Inscription et petit-déjeuner chaud</i>		
7 :20	Organic acids in today's livestock production <i>Usage actuel des acides organiques en productions animales</i>		Dr. Cinta Sol Norel Animal Nutrition
8 :00	Introduction		Marvin Stevenson Halchemix Canada Inc.
8 :10	Factors affecting limestone solubility and its impact on phytase efficacy <i>Facteurs qui interviennent sur la solubilité du calcaire et impact sur l'efficacité de la phytase</i>		Dr. Wenting Li DuPont
8 :50	Natural betaine: function and practical application <i>Bétaïne naturelle : fonction et application pratique</i>		Dr. Janet Remus DuPont
9 :30	L-carnitine for sows: 25 years of research-developed applications <i>La L-carnitine chez les truies : 25 années d'applications nées de la recherche</i>		Dr. Jason Woodworth Kansas State University
10 :10	Break / <i>Pause</i>		
10 :40	Novel products/technologies affecting rumen fermentation and ruminant microbiome / <i>Produits et technologies novateurs intervenant sur la fermentation ruminale et le microbiome des ruminants</i>		Dr. Franklin Evans Acadian Seaplants Ltd.
11 :30	A current understanding of DCAD – an update on reducing hypocalcemia <i>État actuel des connaissances sur la DACA – une mise à jour sur les moyens de réduire l'hypocalcémie</i>		Dr. Jesse Goff Iowa State University
12 :00	Lunch / Visit of student posters <i>Dîner / Visite des affiches produites par les étudiants</i>		

2018 ANCC CONFERENCE PROGRAM

PROGRAMME DU COLLOQUE CNAC 2018

WEDNESDAY, MAY 2ND – AFTERNOON / MERCREDI LE 2 MAI – APRÈS-MIDI

Opening Plenary / Plénière d'ouverture Room / Salle: Salon A & B		
1 :00	Opening remarks from ANAC <i>Propos d'ouverture de l'ANAC</i>	Melissa Dumont ANAC
1 :10	Welcome from organizing committee <i>Mot de bienvenue du comité organisateur</i>	Rob Patterson Canadian Bio-Systems Inc.
1:15	JM Bell Memorial Lecture: Factors affecting energy intake and partitioning for efficient productive performance Conférence commémorative JM Bell : Facteurs influençant la consommation et la répartition de l'énergie pour favoriser l'efficacité de la performance de production	Dr. Michael S. Allen Michigan State University
2 :15	Role of livestock sector in global sustainability <i>Le rôle du secteur de l'élevage dans le développement durable global</i>	Dr. Ermias Kebreab University of California (Davis)
3 :00	Break / Pause	
3 :30	Modeling and integrating metabolizable energy and protein supply and requirements in dry and lactating dairy cattle to optimize nitrogen utilization <i>Modélisation et intégration de l'apport et des besoins en énergie métabolisable et protéines chez les bovins laitiers au tarissement et en lactation pour optimiser l'utilisation de l'azote</i>	Dr. Michael Van Amburgh Cornell University
4 :15	Advances in the understanding of dietary fibre and its components in relation to the use of alternative feed ingredients in modern poultry and livestock production <i>Progrès réalisés dans la compréhension de la fibre alimentaire et de ses composants dans le contexte de l'utilisation d'ingrédients alimentaires de remplacement en production animale aujourd'hui</i>	Dr. Bogdan Slominski University of Manitoba
5 :00	ANAC scholarship announcement and recipient's presentation of research: Effects of pre-weaning iron supplementation on post-weaning disease challenge, growth performance and antibody response in pigs <i>Annonce de la bourse d'études de l'ANAC et présentation de la récipiendaire sur sa recherche : Effets de la supplémentation en fer avant le sevrage sur la santé, la performance de croissance et la réponse immunitaire après le sevrage chez les porcs</i>	Victoria Seip University of Guelph Recipient / <i>Lauréate</i>
5 :15	End of opening plenary / <i>Fin de la plénière d'ouverture</i>	
5 :15 – 7 :15	ANCC 2018 Reception: Come and enjoy an evening of food, drinks and networking around the supplier showcase and graduate student poster competition. Réception du CNAC 2018 : Venez profiter d'une soirée où l'on servira nourriture et boissons, alors que vous aurez l'opportunité de réseauter au salon des fournisseurs et du Concours d'affiches pour étudiants diplômés. Room / Salle: Promenade, Bellamy's Lounge, River Valley	

2018 ANCC CONFERENCE PROGRAM

PROGRAMME DU COLLOQUE CNAC 2018

THURSDAY, MAY 3RD – MORNING / JEUDI LE 3 MAI – MATIN

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

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

7 am Hot breakfast / 7 h Petit-déjeuner chaud

Concurrent Sessions / Séances concurrentes

Monogastric Session / Séance sur les monogastriques		
Room / Salle : Salon A		
8 :10	Opening remarks by Session Chair <i>Propos d'ouverture du Président de la séance</i>	Dr. Mohsen Pourabedin Cargill Animal Nutrition
8 :15	Dietary starch and fiber as prebiotics in swine diets <i>Utilisation de l'amidon et des fibres alimentaires comme prébiotiques dans l'alimentation des porcs</i>	Dr. Ruurd Zijlstra University of Alberta
9 :00	Understanding the needs of branch chain amino acids in broiler production / <i>Comprendre les besoins en acides aminés à chaîne ramifiée en production de poulet à griller</i>	Dr. William (Bill) Dozier University of Auburn
9 :45	Break / Pause	
10 :15	Optimizing utilization of co-products in poultry and swine nutrition <i>Optimiser l'utilisation de coproduits dans l'alimentation des volailles et des porcs</i>	Dr. Martin Nyachoti University of Manitoba
11 :00	Can mammary development be enhanced by nutritional strategies in swine? / <i>Peut-on se servir de stratégies nutritionnelles pour stimuler le développement mammaire chez le porc?</i>	Dr. Chantal Farmer Agriculture and Agri-Food Canada (Sherbrooke)
11 :45	Growth performance of weaned pigs fed raw, cold-pelleted, steam-pelleted, or extruded field pea <i>Performance de croissance des porcelets au sevrage recevant du pois de grande culture brut aggloméré à froid, aggloméré à la valeur ou extrudé</i>	Jill Hugman University of Alberta Graduate student / <i>Étudiante diplômée</i>
12 :00	Lunch / Dîner	

Ruminant Session / Séance sur les ruminants		
Room / Salle : Salon B		
8 :10	Opening remarks by Session Chair <i>Propos d'ouverture du Président de la séance</i>	Dr. Masahito Oba University of Alberta
8 :15	Effective fiber for dairy cattle, how much is not enough? <i>Fibre efficace chez les bovins laitiers, quelle quantité faut-il?</i>	Dr. Paul Kononoff University of Nebraska-Lincoln
9 :00	Walking the tightrope: the balance between immunity and inflammation in ruminants / <i>Le numéro de funambule : maintenir l'équilibre entre l'immunité et l'inflammation chez les ruminants</i>	Dr. Barry Bradford Kansas State University
9 :45	Break / Pause	
10 :15	Risk for ruminal acidosis during finishing <i>Les risques d'acidose ruminale durant la finition</i>	Dr. Greg Penner University of Saskatchewan
11 :00	Fetal programming and its implications for the beef industry <i>La programmation fœtale et ses répercussions pour l'industrie du bœuf</i>	Dr. Philippe Moriel University of Florida
11 :45	Can differing pre- and post-weaning phase nutrition change intake, growth and hasten puberty in Holstein heifer calves? <i>Une alimentation différente avant et après le sevrage peut-elle modifier la consommation et la croissance et hâter la puberté chez les génisses Holstein?</i>	Justin Rosadiuk University of Alberta Graduate student / <i>Étudiant diplômé</i>
12 :00	Lunch / Dîner	

All sessions will be presented in English.

Toutes les sessions seront présentées en anglais.

2018 ANCC CONFERENCE PROGRAM PROGRAMME DU COLLOQUE CNAC 2018

THURSDAY, MAY 3RD – AFTERNOON / JEUDI LE 3 MAI – APRÈS-MIDI

Closing Plenary / Plénière de clôture Room / Salle: Salon A & B		
1 :15	Alberta Animal Nutrition Lecture: Oxidative damage of biological molecules on animal metabolism and physiology Exposé de l'Alberta Animal Nutrition : Dommages oxydatifs des molécules biologiques sur le métabolisme et la physiologie des animaux	Dr. Peter Surai Feed-Food Ltd Scotland
2 :00	Can carbon smart agriculture help the livestock industries meet rising world food and sustainability demands? <i>L'agriculture intelligente face au climat peut-elle devenir une réponse des industries de l'élevage aux demandes mondiales accrues en matière de production alimentaire et de durabilité</i>	Dr. Richard Eckard University of Melbourne
2 :45	Closing remarks <i>Propos de clôture</i>	Dr. Daniel Ganesh Bos Nutrition Services Inc.

ANAC will be hosting a session on Canadian regulatory updates from 3:30-5:00 pm, directly following the Animal Nutrition Conference of Canada.

L'ANAC présentera une séance sur la mise à jour de la réglementation canadienne, de 15 h 30 à 17 h, tout de suite après le Colloque de nutrition animale du Canada.





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Phylogenics
& Probiotics



Grain Management
Technology



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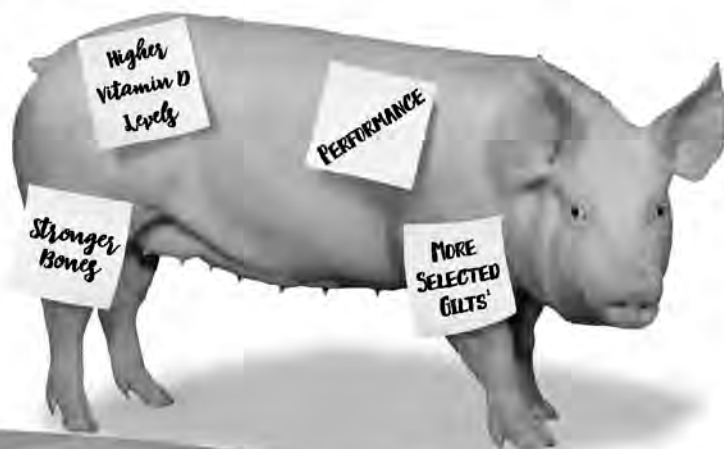


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Organic Acids in Today's Livestock Production

Usage actuel des acides organiques en productions animales

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Abstract

Organic acids have been used for decades as forage and grain preservatives and in livestock nutrition. They make an important contribution to feed hygiene, as they suppress the growth of mold and bacterial pathogens. Due to their antimicrobial activity are also effective in maintaining the nutritional value of the feed to ensure animal performance, as well as improving nutrient digestibility, which in turn leads to stable animal health and increased performance. The most commonly used organic acids in feed are propionic, fumaric, formic and lactic acid, with an overall Compound Annual Growth Rate of 4.5%. Europe represents the biggest market due removal of sub-therapeutic antibiotics in 2006 (Regulation (EC) N° 1831/2003). This situation has increased the interest in improving intestinal health and nutrient utilization. Different feed additives and nutritional strategies have shown promise in changing and mediating the response and recovery of the animals to various stressors and challenges after the AGP's prohibition. The primary effect of antibiotics is antimicrobial and organic acids have several additional effects that go beyond that antimicrobial activity. Organic acids have a clear and significant benefit in weanling piglets and in poultry performances. This review presents recent studies on the effect of organic acids on enteric diseases, nutrient digestibility, immune response and performance in broilers and piglets.

Résumé

Les acides organiques sont utilisés depuis des décennies comme agents de conservation des fourrages et des grains ainsi que dans l'alimentation du bétail. Ils contribuent largement à l'hygiène des aliments, parce qu'ils limitent la croissance des moisissures et bactéries pathogènes. Grâce à leur activité antimicrobienne, ils sont également efficaces pour protéger la valeur nutritive des aliments qui garantit la performance des animaux et pour améliorer la digestibilité des nutriments, ce qui stabilise la santé animale et améliore la performance. Les acides organiques les plus couramment utilisés sont les acides propionique, fumarique, formique et lactique, qui présentent globalement un taux de croissance annuel composé de 4,5 %. L'Europe constitue le plus important marché en raison de l'interdiction visant l'usage sous-thérapeutique des antibiotiques en 2006 (règlement [CE] 1831/2003). Cette situation a stimulé l'intérêt pour une meilleure santé intestinale et une meilleure utilisation des nutriments. Différents additifs alimentaires et stratégies nutritionnelles ont donné des résultats prometteurs pour ce qui est de modifier ou de soutenir la réponse et la récupération des animaux exposés à divers facteurs de stress et provocations depuis que les antibiotiques stimulateurs de croissance ont été bannis. Les antibiotiques exercent d'abord et avant tout une activité antimicrobienne, et les acides organiques offrent plusieurs autres atouts

outre l'activité antimicrobienne. Les acides organiques ont un avantage clair et net chez les porcelets au sevrage et pour la performance des volailles. Cet exposé présente les études récentes réalisées sur l'effet des acides organiques sur les maladies entériques, la digestibilité des nutriments, la réponse immunitaire et la performance chez les poulets à griller et les porcelets.

Introduction

The European Union allowed the use of organic acids and their salts in animal production because these are generally considered safe (Adil et al., 2010). Formic, propionic, lactic, citric, fumaric and sorbic are used under the classification “feed preservative” (Lückstaadt and Mellor, 2011) and have been included for decades in feeds. Other short-chain fatty acids (C4-C7) or medium-chain fatty acids (MCFA, C8-C12) are feed materials. Some of these acids are synthetics and others like MCFA's are naturally present in foods such as coconut oil and palm kernels.

They are characterized by the possession of one or more carboxylic group with general structure of R-COOH. Their capacity to give up protons defines how strong or weak is an acid. A quantitative measure of acid strength is pK (a negative logarithmic scale of dissociation constant). It is characteristic of each acid and depends on whether it is lineal (formic, acetic, propionic), hydroxyl group contents (lactic, malic and citric acid) or it has the presence of double bonds like fumaric and sorbic acids (Shahidi et al., 2014).

Organic acids are available on the market in a variety of forms:

Adsorbates: liquid acids or mixtures of acids adsorbed onto a solid, inert substrate, usually silica or sepiolite.

Salts: produced by reaction acid-base to obtain solid product (except, for example, ammonium formate and ammonium propionate which are liquids) to minimize functional properties such as corrosion, volatility and odor.

Coated: produced by mixing with vegetable fat and spray-system with the objective to release the organic acid in the lower gastrointestinal tract.

Protected: produced by double reaction of organic acid and medium or long chain fatty acids delaying the release of carboxylic acid along the gastrointestinal tract.

This review presents recent studies on the effect of organic acids on enteric diseases, nutrient digestibility, immune response and performance in broilers and piglets.

Microbiological Effect

Molds are present in feed ingredients and will grow when environmental conditions allow. It's important to ensure they are controlled in order to avoid deterioration of feeds. The addition of propionic acid is suitable onto high moisture whole cereal grain where mold growth is very likely and a rapid control measure is needed. Combining different short chain fatty acids (e-g.

propionic in combination with acetic or formic acid) is known to enhance the mold-reducing effect of individual acids (Fefana, 2014).

Salmonella, *Campylobacter* and *Escherichia coli* are the most common bacteria that affect the intestinal health and are important for public health.

Odd-numbered medium-chain fatty acids (MCFA), such as C7 and C9 could have potential as feed additives but are poorly described. In a *in vitro* digestion test both compounds showed a significant reduction of analyzed bacterial count (*Coliforms*, *Streptococci*, *Lactobacilli*) in the stomach and small intestine (De Smet et al., 2016).

In challenging tests in poultry with *Salmonella*, feed supplementation with acetic and formic acid resulted in an increase colonization of ceca, liver and spleen while birds receiving propionic acid as feed supplement were colonized to the same extent as control group. However, butyrate in feed, resulted in a significant decrease of colonization by *S. enteritidis* in the ceca but not in internal organs (Van Immerseel, 2014).

Protected sodium butyrate demonstrated a significant reduction of *Salmonella* infection in birds with better results than non-protected presentation (Fernández-Rubio et al., 2009). In a similar way, different presentations of butyrate (protected with sodium salts of palm fatty acids distillates or coated with vegetable fat) and protected sodium heptanoate showed a significant reduction of *Salmonella* presence in internal organs (spleen and liver) of birds challenged with *Salmonella* (Puyalto et al., 2016).

The dietary administration of protected sodium butyrate during the whole fattening pigs period was able to reduce significantly the seroprevalence and shedding of *Salmonella*, which may reflect a positive effect on the control of *Salmonella* at the end of this period (Casanovas-Higes et al., 2017a) (Figure 1).

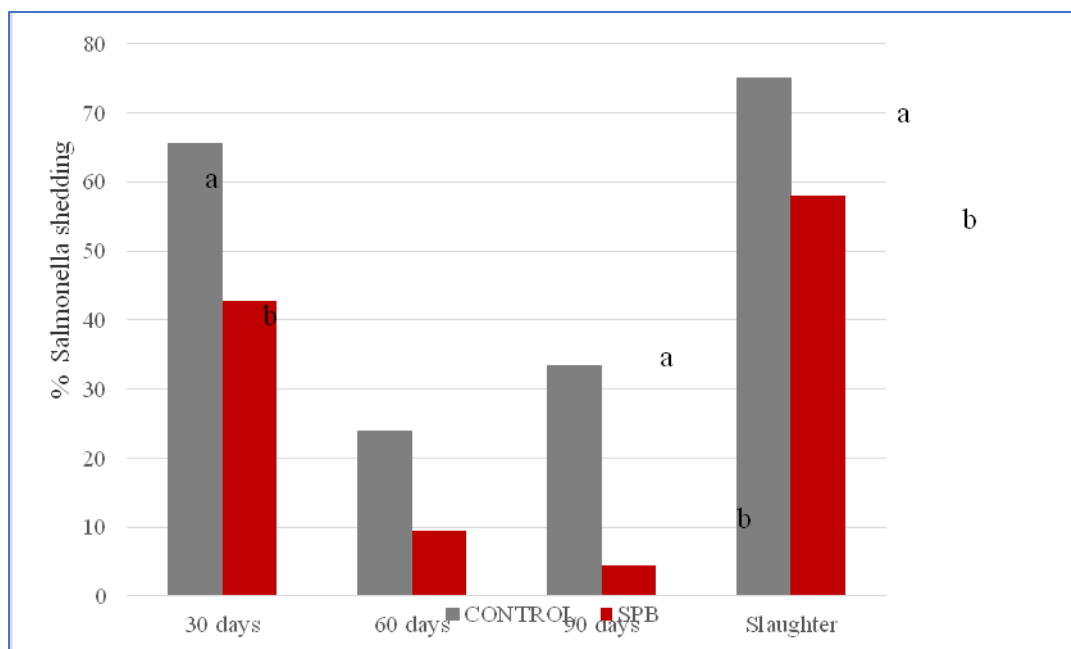


Figure 1. Sodium Butyrate in Protected form reduce the Salmonella shedding in fattening pig farm

Organic acids can be used in combination with MCFA. MCFA have larger molecules compared with organics acids with slower breakdown and with the capacity to reach the lower part of the intestinal tract where can exert an antimicrobial effect, while organic acids (with no protection or encapsulation) are present at the beginning of the small intestine. The inclusion of sodium salts of coconut fatty acids distillates (3 Kg/t) with high level of MCFA (mainly C12) in swine, significantly reduced coliforms in ileum and in caecum (Sol et al., 2016). In addition, MCFA are effective against Gram + bacteria, such as *Clostridium perfringens* (Sol et al., 2017). It has been demonstrated that, a new form of sodium butyrate protected with sodium salt of coconut fatty acid distillate (3 Kg/t) controls *Salmonella* infection in fattening pigs with a significant reduction in the number of infected pigs (4% vs. 61%; $P < 0.01$). The median OD% value for both groups was similar at 30 days, but in subsequent samplings median OD% values remained significantly lower for the group supplemented with the additive (Figure 2) (Casanovas-Higes et al., 2017b).

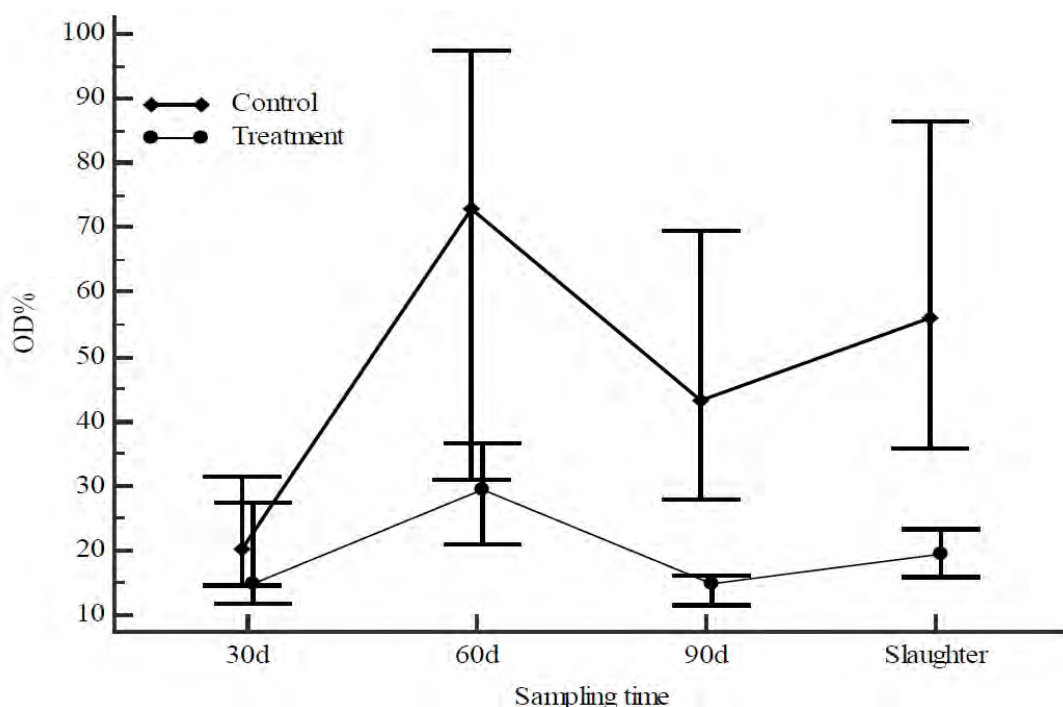
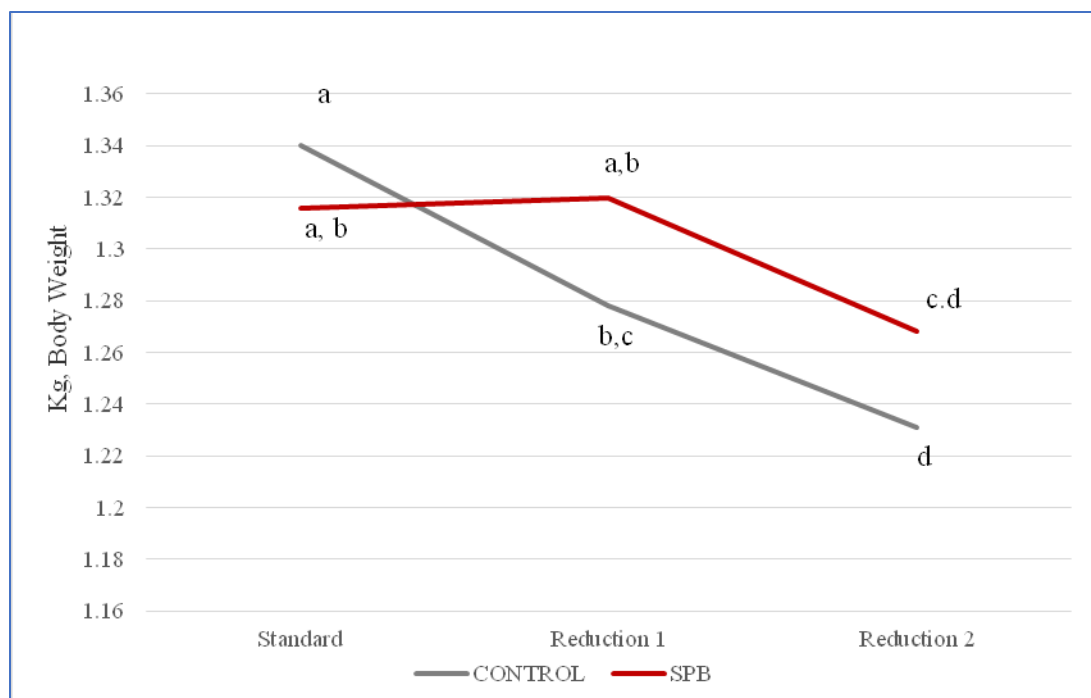


Figure 2. Median OD% values for the control and treatment group (sodium butyrate protected with sodium salts of coconut fatty acids distillate) in fattening pigs

Nutrient Digestibility Effect

Organic acids are an option for improving nutrient digestibility. In poultry diets, dietary 0.5% fumaric or 2% citric acid increased digestibility of CP and EE and 0.5% of fumaric acid or 2% citric acid increased ME (Ghazala et al., 2011). Moreover, supplementation with 0.5% or 1% formic acid (Hernández et al., 2006 and García et al., 2007) in broiler finisher diets was found to improve apparent ileal digestibility (AID) of dry matter (DM; 67.8% or 68.8%, respectively) and crude protein (CP; 72.5% or 73.5%) as compared with control (56.4% DM and 60.7% CP). Lohakare et al. (2005), found at 19 days that gross energy (GE), CP and ether extract (EE) in supplemented group with 2% ascorbic acid was higher than non-supplemented group. The addition of sodium butyrate at 1 Kg/t of feed, showed a significant digestibility improvement of energy (5.8%) and protein (4.7%) in broilers (Mallo et al., 2011). It may be related by the better development of the intestinal epithelium showing an increased intestinal surface that was in contact with the feed bolus ensuring a better digestion (Pluske et al., 1997). This diet digestibility improvement may explain the reduction in feed conversion ratio that is normally observed with the use of sodium butyrate (Mallo et al., 2011). Considering those facts, it was designed a trial to prove, that the addition of protected sodium butyrate could reduce the energy and protein content of the diets without negative effects on performance of broilers. The results showed that sodium butyrate in protected form (1 Kg/t) improved the performance of nutritionally reduced feed broiler (- 60 and 120 Kcal of ME and - 2.3% and 4.6% of total amino acids) (Bortoluzzi et al., 2017).



Reduction 1: - 60 Kcal of ME and - 2.3% of total amino acids
Reduction 2: - 120 Kcal of ME and - 4.6% of total amino acids

Figure 3. Body Weight at 28 days of age, fed diets with different levels of reduction in energy and AA, and supplemented or not with protected sodium butyrate (SBP)

Effect on immune response

Organic acids may stimulate the natural immune response in poultry and swine. Houshmand et al. (2012) found that at 21 days of age of broilers, dietary addition of organic acids (combination of Formic, Lactic, Malic, Tartaric, Citric and Orthophosphoric acid. (0.15% starter diet) resulted in significant increases in antibody titres against Newcastle disease. Abdel-Fattah et al. (2008) and Ghazala et al. (2011) reported that birds fed an organic-acid-supplementation diet (formic or acetic or citric or fumaric) had heavier immune organs (bursa of Fabricius and thymus) and higher level of globulin in their serum. Concentration of globulins is used as an indicator for measuring immune response. Citric acid supplementation (0.5%) in poultry diets enhanced the density of the lymphocytes in the lymphoid organs, enhancing the non-specific immunity (Haque et al., 2010).

Dietary substances can manipulate the expression of endogenous host defense peptides (HDPs), which may provide a promising strategy for disease control and prevention, especially for antibiotic-resistant infection. Feed supplementation with 0.1% butyrate led to a significant increase in HDP gene expression in the intestinal tract of chickens (Sunkara, et al. 2011).

Butyrate in piglets (Xiong et al., 2015) enhances disease resistance promotes clearance of *E. Coli* O157:H7 and alleviates clinical symptoms of hemolytic uremic syndrome and inflammation partially by affecting endogenous HDP expression.

Performance Effect

The multifactorial analysis of Partanen et al. (2001) demonstrated that organic acids improved all performance parameters in weaned and fattening pigs compared to non-acidified control diets.

In 2008, it was applied to a dataset collected from all available published material on the use of acidifiers in pigs using 458 publications and comprising nearly 38,000 pigs (Rosen, et al., 2008). Most of these tests included fumaric, citric, formic or propionic acids, calcium formate, potassium diformate and propionic salts and were performed with weaners. The holo-analytical models derived demonstrate that using acids in pig diets improves the productivity parameters of greatest importance to economic success. The magnitudes of improvements were: 1.2% on feed intake, 5.5% on weight gain and 3.7% on feed conversion ratio (FCR).

On the other hand, the addition of 0.4% of butyrate in broiler diets was similar to antibiotics in maintaining body weight gain (Panda et al., 2009). The use of sodium butyrate protected with sodium salt of palm fatty acids and Zinc Bacitracin combination, resulted in a significant improvement of FCR when compared with the control treatment without additives (Ortiz et al., 2014). In other study, broilers fed with sodium butyrate in protected form showed significantly higher EPEF (European Production Efficiency Factor) than animals receiving the control diet (Mallo et al., 2010). Chicks fed with organic acids (3% Fumaric or 3% Lactic acid) showed a significant improvement in the FCR versus the control diet (Adil et al., 2010 and 2011b) found that the highest weight gains were achieved in the birds fed fumaric compared to lactic acid. Another trial in which broilers were given basal diet supplemented with 2-3% each of butyric, fumaric and lactic acid (Adil et al., 2011a) showed a significant improvement in FCR as against the chicks fed the control diet. Fascina et al. (2012) reported that the use of an organic acid mixture (comprising 30% Lactic acid, 25.5% benzoic acid, 7% formic acid, 8% citric acid and 6.5% acetic acid) in broilers diets improved its performance as compared to the control at 42 days. An increase of body weight gain was observed in broilers fed with added and acidifier mixture (formic, phosphoric, lactic, tartaric, citric and malic acids) in the broiler diet at the rate of 0.15%.

Conclusion

Organic acids and their salts are active ingredients available for the feed. Their effects are far beyond the control of mold and bacteria in the feed and are basic for the new age of production without the use of medicines. Which organic acid or combination, their presentations and doses, depend on the species, environment and diet formulation among other parameters, and every nutritionist must think, evaluate and find which is the best solution for their situation.

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Factors Affecting Limestone Solubility and its Impact on Phytase Efficacy

Facteurs qui interviennent sur la solubilité du calcaire et impact sur l'efficacité de la phytase

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Abstract

Limestone, the predominant Ca source in poultry feeds worldwide, can have particle sizes that are widely different, from less than 75 μm to more than 800 μm , which will impact the concentration of reactive (soluble) Ca. There is a well-documented detrimental impact of Ca on P digestibility, but the impact of limestone particle size which is highly correlated with its solubility and is a key determining factor on the degree of impact of Ca from limestone on P utilization, has been less studied. Limestone currently used in animal production varies considerably in particle size, purity and solubility. Neither of these three parameters by itself dictate the availability of Ca for animals or the impact of Ca on the digestibility of other nutrients (e.g. phosphorus). The variation in Ca solubility must be considered to properly assess the interaction of Ca with phytate and phytase and to formulate diets for optimal animal performance.

Résumé

La taille des particules de calcaire, principale source de calcium (Ca) utilisée à travers le monde dans l'alimentation des volailles, peut varier grandement – moins de 75 μm à plus de 800 μm –, ce qui modifiera la concentration de Ca réactif (soluble). L'effet préjudiciable du Ca sur la digestibilité du phosphore (P) est bien documenté, mais l'impact de la taille des particules de calcaire, une caractéristique fortement liée à sa solubilité et un des principaux facteurs déterminant le degré d'influence du Ca du calcaire sur l'utilisation du P, a été moins étudié. La taille des particules, la pureté et la solubilité du calcaire présentement utilisé en production animale varient considérablement. Aucun de ces trois paramètres n'est à lui seul responsable de la disponibilité du Ca pour les animaux ni de l'impact du Ca sur la digestibilité des autres nutriments (p. ex., P). La variation de la solubilité du Ca doit être prise en considération pour évaluer correctement l'interaction entre le Ca et le phytate / phytase et formuler des rations pour optimiser la performance animale.

Introduction

Practical plant seed based poultry diets are normally deficient in Ca and require the addition of concentrated Ca sources to meet the Ca requirement for growth. Among all the Ca sources,

limestone, a naturally occurring mineral primarily composed of CaCO_3 , is the predominant Ca supplement used in broiler production world-wide. However, considerable variations in limestone Ca solubility and Ca concentration have been reported even within the same country or region and preference of limestone particle size differs among countries/regions. In addition to its nutritional property, limestone is also widely used as flow agent (eg. premixes and feed ingredients) and diluting material (eg. ionophores, premixes, enzymes and antibiotics) in the feed industry, the Ca of which may not be accounted for in the feed formulation.

It is well documented that Ca has a detrimental impact on phytate P (PP) utilization in broilers, mainly through the formation of Ca-PP complexes, especially when high Ca with severely deficient non-phytate P (nPP) diets are fed. Even though several studies have been published where the impact of Ca particle size on P utilization was evaluated, results have not been conclusive. This could be partially due to the confounding effect from either source differences in Ca (limestone, egg shell, etc.) and/or origin of the different limestones being tested as well as particle size/solubility of the Ca used.

Limestone is inconsistent in particle size and solubility

Solubility of limestone is highly correlated with particle size. In general, finer particle size is an indication of more rapid and higher solubilization. In other words, finer limestone will release more Ca^{2+} in a defined period of time under acidic conditions ($\text{pH} < 6$) as compared to those coarser ones and consequently has higher impact on phytate availability. However, there is increasing evidence that suggests the average particle size alone, described as geometric mean, may be insufficient for solubility determination. In 2017, DuPont Animal Nutrition did a pilot survey on 9 commercial limestone samples and found the average particle sizes (geometric mean) ranged from 63.26 to 495.18 μm , with standard deviations being 50 to 95% of the particle size. In addition, those limestones with similar solubility do not necessary share similarity in the particle size or vice versa (Figure 1). These results clearly suggest that other factors, such as particle size distribution and chemical composition need to be considered together with particle size to properly evaluate the solubility of limestone samples.

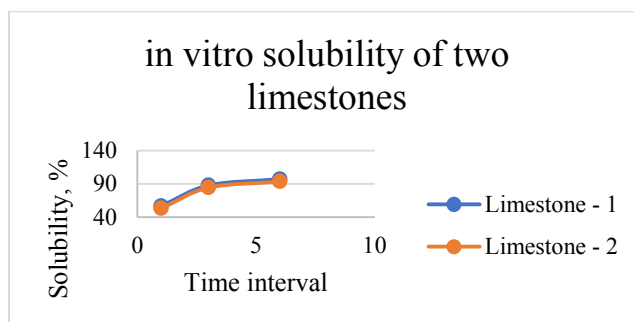


Figure 1. *in vitro* solubility comparison of two limestone from survey (DuPont, unpublished). Solubilization does not defined by particle size alone.

Limestone – 1: geometric mean = 495.18 μm , standard deviation = 265 μm .

Limestone – 2: geometric mean = 265 μm , standard deviation = 278 μm .

Evaluating the impact of limestone particle size on P digestibility and phytase efficacy

In an experiment conducted by Kim et al. (unpublished), the researchers examined the impact of limestone particle size on P digestibility and phytase efficacy. To avoid composition differences, one commercial limestone was purchased and a well-mixed subset was obtained from various bags of the same batch (particulate limestone, PAL) with a geometric mean diameter of 402 μm . Then an aliquot of the well-mixed subset was taken, and ground through a 75 μm mesh (pulverized limestone, PVL). The geometric mean diameter of the PVL was smaller than 75 μm .

The PVL limestone, due to the very fine particle size, solubilized instantly *in vitro*, whereas at the end of 10 min incubation, the solubility of PAL limestone was still 10 percentage points lower compared to PVL (Figure 2). In a subsequent animal trial, the PVL also showed greater impact on gizzard pH, with an average pH of 2.43 compared with PAL (average=2.27, $P<0.05$). Consequently, P digestibility was 28% lower and phytase efficacy was also reduced in the PVL group (Figure 3) as the result of feeding fine particle size limestone in broiler chickens.

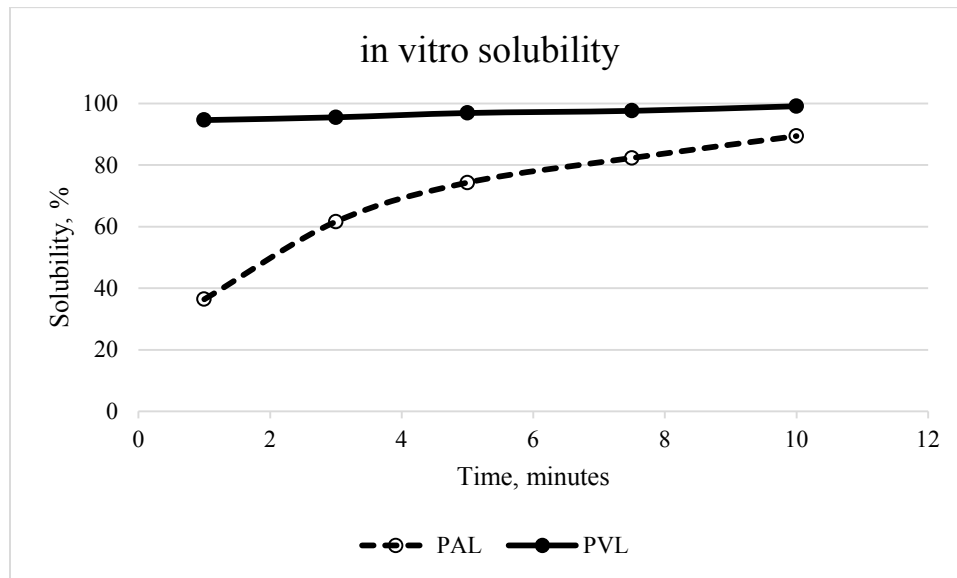


Figure 2. *in vitro* limestone solubility at different time, at pH=0.7 (adapted from Kim et al, unpublished). PAL: average limestone particle size = 402 μm ; PVL: average limestone particle size = 75 μm .

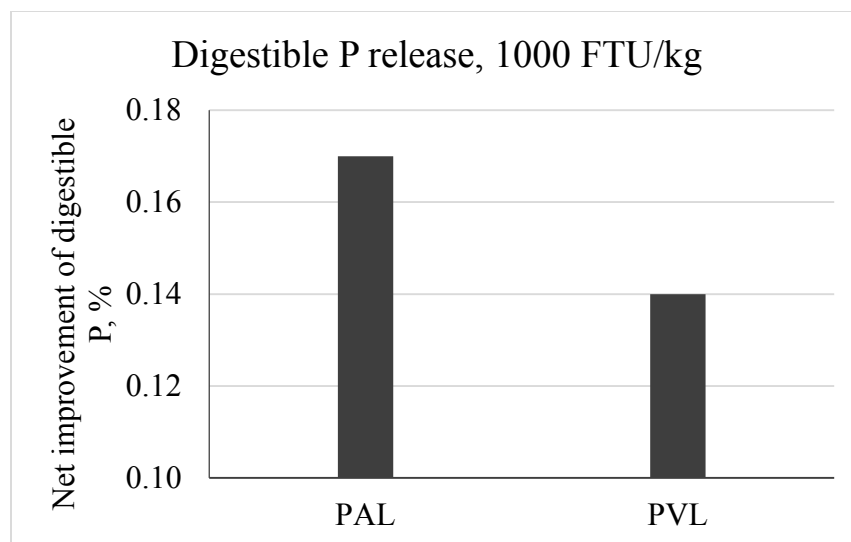


Figure 3. Phytase efficacy expressed as digestible P release compared to control without phytase (adapted from Kim et al, unpublished). PAL: average limestone particle size = 402 μm ; PVL: average limestone particle size = 75 μm .

However, it needs to be noted that while larger particle size limestone tends to dissolve slower, less complete, and have less impact on phytate availability, there appears to be an optimal size beyond which limestone particles become too large. The optimum limestone particle size for bone mineralization and body weight gain in 28-d old broiler chicken was reported to be between 137 and 388 μm (Manangi and Coon, 2007). In their study, particle size greater than 800 μm was also found to be detrimental for performance. This observation certainly highlighted the need to better understand the correlation between *in vitro* limestone solubility and *in vivo* availability, and the impact on digestibility of other nutrients.

References

Manangi, M.K., and C.N. Coon. 2007. The Effect of Calcium Carbonate Particle Size and Solubility on the Utilization of Phosphorus from Phytase for Broilers. *Poult. Sci.* 6:85-90

Natural Betaine: Function and Practical Application

Bétaïne naturelle : Fonction et application pratique

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Abstract

Natural betaine can be found in a wide variety of plant and animal species albeit it at widely varying levels. In living systems, natural betaine, a charge-neutral zwitterion readily capable of hydrogen-bonding with water, is used as an osmolyte to aid in maintenance of cellular water balance and as a methyl group donor. Betaine also can serve as a chemoattractant in some marine species. While vertebrates have a limited ability to produce betaine within the body, this does not affect the response to supplemental natural betaine. In its methyl role for vertebrates, natural betaine donates a methyl group to homocysteine via an enzymatic reaction in the liver's transmethylation pathway to form methionine, this allows the body to recycle the nonessential/nonstructural amino acid homocysteine back to methionine. Natural betaine's osmolyte role helps minimize water loss from cells, tissue or the body particularly during periods of dehydrating stresses. Research has documented responses to natural betaine in diverse areas such as hydration during osmotic stress, villus height in coccidia-challenged birds, breast meat yield in poultry, energy utilization and litter size in swine. Regardless of the measured parameter, all responses seen with natural betaine relate to one or both of its physiological functions.

Résumé

On trouve la bétaïne naturelle chez une grande variété d'espèces végétales et animales, bien qu'à des concentrations très variables. Dans les systèmes vivants, la bétaïne naturelle, un zwitterion de charge neutre capable de créer facilement des liaisons hydrogène avec l'eau, est utilisée comme osmolyte pour aider à maintenir l'équilibre hydrique des cellules et comme donneur de groupe méthyle. La bétaïne peut également servir d'agent chimiotactique chez certaines espèces marines. Bien que les vertébrés ne produisent que peu de bétaïne, cela n'empêche pas la réponse à la supplémentation en bétaïne naturelle. Dans son rôle de méthylateur chez les vertébrés, la bétaïne naturelle donne un groupe méthyle à l'homocystéine par le biais d'une réaction enzymatique dans la chaîne de transméthylation hépatique pour former de la méthionine, un phénomène qui permet à l'organisme de recycler l'homocystéine, un acide aminé non essentiel / non constitutif, en méthionine. En tant qu'osmolyte, la bétaïne naturelle aide à réduire au minimum la perte d'eau par les cellules, par les tissus et par l'ensemble de l'organisme, particulièrement en période de stress hydrique. La recherche a documenté les réponses à la bétaïne naturelle pour divers paramètres, telles qu'hydratation pendant un stress osmotique, hauteur des villosités chez des oiseaux exposés aux coccidies, rendement en poitrine chez la volaille, utilisation de l'énergie et

taille des portées chez les porcs. Quel que soit le paramètre mesuré, toutes les réponses observées avec la bétaine naturelle sont associées à au moins une de ses deux fonctions physiologiques.

Introduction

Natural betaine, aka trimethylglycine, has two physiological functions: 1) osmolyte and 2) methyl donor. An osmolyte can be described as that affects the movement of water, is taken up by the cell quickly, does not have any adverse impact on the cell and protects cellular macromolecules from inactivation. There are many substances capable of affecting water movement, however the vast majority are used by the cell for other purposes. Betaine, on the other hand, can be used as an osmolyte by all the tissues in vertebrates although the liver is typically the only tissue that can both demethylate betaine and use it as an osmolyte.

The Osmolyte Role

To understand why betaine's osmolyte role is important physiologically, it must first be understood that water content of the cell is a key factor in determining what physiological state the cell is in. The goal of osmotic regulation is to keep the cell volume as constant as possible (Haussinger, 1996). However, change in cell volume can cause a change in cell activity. A slightly swollen state is characteristic of an anabolic state or growth mode of the cell. In contrast, a slightly dehydrated state indicates a catabolic state or "degrading/break-down" mode. Electrolytes and osmolytes like betaine are not the only factors that influence cell hydration.

Hormones such as insulin and glucagon can as well (Haussinger, 1996). Although some changes in cell water level are normal, large changes are stressful and potentially harmful to the cell's long-term survival. This means that cell must find efficient ways of coping with these problems when they arise. Betaine is particularly useful to cells when they are in danger of dehydration (beyond "normal" parameters).

Hydration is more than a cellular issue, it is a whole animal challenge and one that can cost in terms of maintenance energy. Consider that 98% of all the molecules in the body are affected by water (NRC Beef Cattle, 1996). The body and its tissues are rich in water held within cells and in the extracellular space:

- Y 1.5 kg neonatal piglet at empty (no GI tract) body weight is 82% water (Shields et al, 1983)
- Y 90 kg to 110 kg market hog is 48 to 53% water (empty body weight basis) (Shields et al, 1983; de Lange et al., 2001)
- Y 56 to 81% of body weight is water in dairy cattle depending on age and stage of production (Murphy, 1992)
- Y Broiler fryer is 66% water (USDA, 2011)
- Y Muscle is 75% water (USDA, 2011)

Hyperosmotic stress causes dehydration of cells. This sort of stress can come from a range of situations including digestion of feed, heat stress and coccidia challenge. Cells have no direct

way to hold or control water movement, so water can move according to the concentration gradient prevailing at that time. When water is lost from cells, the electrolyte level within the cell is correspondingly increased. This increase in concentration results in increased potassium/electrolyte interaction with cellular organelles, proteins and enzymes (Burg, 1994; Petronini *et al.*, 1992; 1994). Since potassium can bind to the active sites of enzymes and other electrolytes destabilize proteins, increases in electrolyte levels become more and more difficult for the cell to handle as the water balance stress continues. However, if the cell cannot control its water loss, it will eventually die.

Because water is a crucial cellular component, nature has devised ways of manipulating water movement. The most commonly recognized method is through use of ion pumps (Figure 1). These pumps act to increase electrolyte levels (potassium mainly) in the cell, the goal is to make the cell more concentrated than its hyperosmotic environment so that water movement will be minimized.

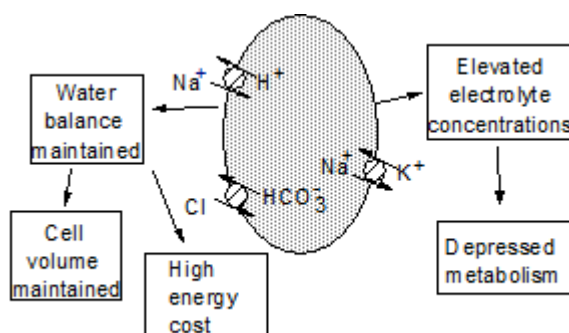


Figure 1. Hyperosmotic stress, compensation with ion pumps

The advantage for the cell is that these pumps do help maintain water balance, however these pumps work best for short-term stresses. For every electrolyte pumped into the cell, energy as ATP is consumed. This means that ion pumps are expensive energetically for the cell to use. In addition, high electrolyte levels lead to problems within the cell as described previously. The most sensitive organelle is the mitochondria, which becomes increasingly compromised with rising electrolyte levels (Björköy 1991). Betaine helped protect mitochondrial respiration despite osmotic stress (Björköy 1991). Since this organelle is the “energy supply house” for the cell, loss of mitochondrial enzyme activity will ultimately affect the ability to make ATP unless a compatible osmolyte like natural betaine is present. The longer the stress goes on, the more problem the cell has meeting the demands of the ion pumps considering the decline in energy production due to electrolyte interference. A classic example of this challenge comes from the historic morbidity and mortality problems that arose from the abrupt transfer of salmon smolts from freshwater tanks to seawater grow-out cages. Evidence on the improvement seen with betaine use can be seen in works such as that of Castro *et al.*, 1998.

An alternative way for cells to control water movement is to use betaine. Accumulation of betaine may take place either by synthesis or uptake of dietary betaine. Uptake seems to be the primary mechanism to obtain betaine for osmoregulation in vertebrates (Björköy 1991, Burg 1994), since kidney and liver are the only tissues that can produce betaine. However, the betaine produced by these tissues is used there, so it is not available for use in other tissues. Dietary

betaine can be taken up by diffusion, however passive or active transport provides a means of concentration in the cell beyond that in its environment. Transport of betaine into the cell can be coupled to diffusion of Na^+ and Cl^- across the cell membrane (Burg, 1995). This means that uptake of betaine increases when fluid osmolarity increases. Two specific carriers for betaine have been documented, namely amino acid transport system A (deAngelis *et al.*, 1999) and betaine-gamma-aminobutyric acid transporter 1 (Takenaka *et al.*, 1994). These carriers concentrate betaine in the cell, so that the level within the cell is higher than outside of the cell (Figure 2).

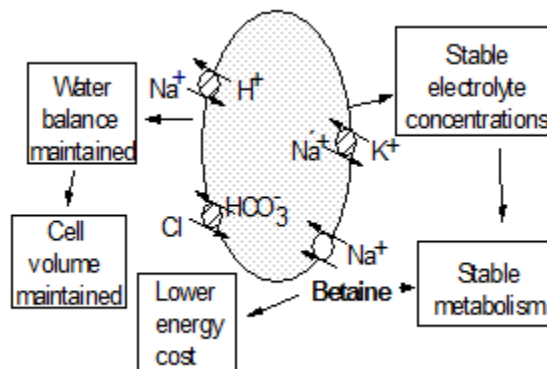


Figure 2. Hyperosmotic stress, compensation with ion pumps and betaine

This concentration difference attracts water and allows the cell to “hold” water during adverse situations. The advantage of betaine for the cell is that betaine does not interfere with enzyme activity (Pollard and Wyn Jones, 1979), so cellular energy production is unaffected. In addition, cellular accumulation of betaine is less costly energetically than electrolytes.

The Methyl Role

So, what about the methyl donor role? Betaine has three methyl groups on its structure. The first of these groups is donated to homocysteine in its enzyme-induced conversion to methionine in the Transmethylation Cycle (Figure 3) of the liver.

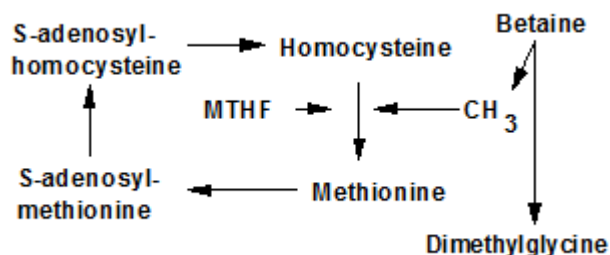


Figure 3. Transmethylation role of betaine in the liver

The other two methyl groups on betaine are donated to the one carbon pool that methylates substances like folic acid. Incidentally, the alternative or “back-up” step for conversion of homocysteine to methionine uses activated folic acid (methyltetrahydrofolate, MTHF) and

vitamin B12 as cofactors. The presence of two separate enzyme systems for the same reaction indicates how important this step is to metabolism. In chickens, Saunderson and MacKinley (1990) noted that the folate based system is less active than the betaine utilizing system, thus the liver folate system could be considered a safety measure in case of low availability of betaine.

Research has shown that about 15% of the methionine level or about 10% of TSAA can be spared in practical trial conditions using U.S. or European type broiler diets (Virtanen and Rosi, 1995). Practically speaking, the amount of methionine that can be spared in any given broiler diet is affected by three factors: bird stress level, dietary cystine level and amount of methionine needed to support nonmethylation functions.

Floor pen research has indicated that the FCR response to betaine supplementation in a basal low methionine diet is affected by the level of stress. This suggests that there is a change in activity of the transmethylation cycle. Since S-adenosylmethionine (S-AM) is a metabolic methyl donor in a variety of reactions, including tissue repair and upregulation of the immune system, it would be logical stress could affect S-AM demand. Indeed, Tiihonen and coworkers (1997) found that birds challenged with a mixture of coccidia had more S-AM in their livers at 21 days (7 days postchallenge) than did nonchallenged broilers (Figure 4).

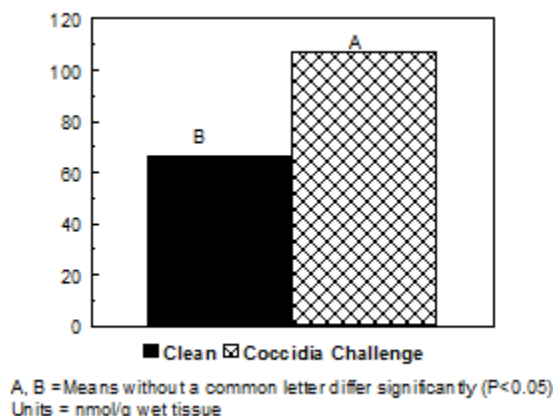


Figure 4. Shifts in S-AM in the liver of broilers with or without coccidia challenge

But why does stress affect the methyl donor role of betaine? By increasing the body's demand for S-AM, stress also increases the level of homocysteine available to be enzymatically methylated back to methionine via a methyl group from betaine.

Homocysteine has several fates in the body. It can be methylated to methionine, converted to cysteine or be excreted on a limited basis. It is, however, an amino acid that can have adverse consequences if it accumulates. Lever and Slow (2010) reviewed the methyl role of betaine in homocysteine conversion to betaine and highlighted areas impacted which ranged from cardiovascular disease to pregnancy.

Practically speaking, conversion to cysteine has a direct impact on betaine activity since it affects the amount of homocysteine available to be methylated. For methionine to be converted to cysteine, it must first go through the methylation cycle to homocysteine (Figure 5). Then homocysteine is combined with serine, which eventually leads to the formation of cysteine.

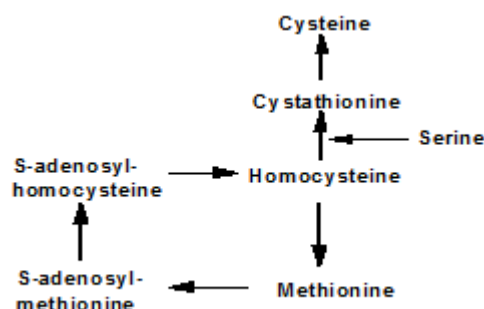


Figure 5. Transsulfuration and Transmethylation relationship

The amount of cysteine that must be made from methionine via homocysteine is dependent how much cystine the diet contains. If the diet is low in cysteine or is poorly digestible, then the amount that must be made from methionine is increased. The reverse is also true, if dietary cystine levels are high or highly digestible, then synthesis from methionine is decreased. Since homocysteine is permanently lost from the Transmethylation pathway when cysteine is formed, diets low in dietary cystine affect betaine activity by decreasing availability of homocysteine for methylation. Research by Emmert and coworkers (1996) shows that cysteine increases the activity of betaine-homocysteine methyltransferase. The implication of this work is that dietary cystine can decrease cysteine synthesis in the liver, which would subsequently increase homocysteine availability and ultimately, the activity of betaine in methylation. To test this, broilers were fed diets containing varying levels of cystine as a percentage of total sulfur amino acids in the presence and absence of betaine. Methionine was held constant in each phase at levels adequate to support nonmethylation functions of this amino acid. In addition, stress level was low in this trial. Built-up litter was used, but no coccidia challenge was present. The results of show that the FCR response to betaine supplementation increased as dietary cystine level increased (Firman and Remus, 1999). Additionally, supplementation of betaine to the moderate and high TSAA diets (cystine used to increase TSAA instead of methionine) resulted in feed:gain values similar to that of the “typical” methionine diet.

The work of Mudd and coworkers (1975; 1980) indicates that amount of methionine needed in the Transmethylation Cycle far exceeds what the dietary consumption is. From an evolutionary standpoint, the methyl-based conversion of homocysteine back to methionine increases the efficiency of methionine use in this pathway, thus lowering the dietary demand from what it could be without this conservation step. However, the methylation of homocysteine does not completely offset the need for methionine in the diet. Livestock diets may contain too low a native methionine level to support nonmethyl functions (i.e. lean tissue accretion); hence some methionine supplementation may be needed even in the presence of betaine. This need for methionine for nonmethyl functions can be termed ‘essential methionine’.

Since choline must be converted to betaine before use in methylation, choline is clearly not 100% bioefficient in methylation. In addition, choline is used in phospholipids and acetylcholine, functions in which betaine does not participate. The reverse is true as well – choline cannot act as an osmolyte in cells. The majority of choline is already bound as phospholipids when it emerges from the gastrointestinal tract of the broiler (Kettunen *et al.*

2001b). Since only free (nonbound) choline can be converted to betaine, the low level of free choline limits the amount of betaine that can eventually be formed. In addition, the conversion from choline to betaine varies in efficiency across species. Work by Saarinen and coworkers (2001) shows that efficiency of choline conversion to betaine is about 55% in the Ross broiler when increase in liver betaine is used as a measure of efficiency.

Choline's methyl role can be taken on by other methyl donors as long as its specific roles in phosphatidylcholine and acetylcholine are satisfied by native ingredients (NRC, 2012). Choline can also be synthesized via methylation of phosphatidylethanolamine. As the active form of choline for methyl group donation, betaine is directly available to transmethylation in the liver. Thus sparing choline's methyl role with betaine is one way of improving methyl efficiency in livestock. In grower/finisher swine, betaine helped to improve weight gain of pigs while an equimolar addition of choline had no effect on pig performance (Siljander-Rasi et al., 2003). Adult poultry can synthesize choline in contrast to very young poultry as long as sufficient methyl groups are present (NRC, 1994). Work by Ferket and coworkers (1993) indicates that betaine can be used instead of choline when the essential requirement of 1300 ppm total choline is met in starter poult diets. Broiler chicks require 450 ppm available choline in the presence of betaine (Lowry et al., 1987). Work by Dilger and coworkers (2007) shows broiler chicks require 10.5 mg choline/d (412 ppm available choline) when betaine is supplemented versus 20.8 mg choline/d (722 ppm available choline) when betaine is not provided. Therefore, the authors conclude that a minimum of 150 ppm available choline is needed for phosphatidylcholine and acetylcholine synthesis and betaine can spare 50% of the available choline requirement in the young broiler.

Natural Betaine in Livestock

In broilers, the first evidence of betaine's osmolyte activity came from coccidia-challenge studies where betaine improved lesion scores of broilers exposed to *Eimeria tenella*, *E. acervulina* and *E. maxima* (Virtanen and Rosi, 1995; Remus et al., 1995; Augustine et al., 1997). Work by Kettunen and coworkers (2001c) noted improved crypt to villus ratio in both coccidia-challenged and nonchallenged broilers. Increased villus height has been seen in broilers (Klasing et al., 2002) and piglets (Slade et al., 2004). Additionally, the crypt:villus area and muscle depth increased in nursery pigs supplemented with natural betaine (Slade et al., 2004). Increased intestinal tensile strength has been noted with betaine supplementation in broilers (Remus and Quarles, 2000), piglets (Slade et al., 2004) and market hogs (Siljander-Rasi et al., 2003). Electron microscopy of duodenal tissue of birds supplemented with betaine appeared less electron dense vs. the control tissue, suggesting a higher water content (Augustine and Danforth, 1999). All of these results are indicative of a more functional intestinal tract, something supported by improvements in the apparent digestibility of lysine, protein, fat and carotenoids (Remus et al., 1995), *in vitro* methionine uptake (Augustine and Danforth, 1999) and feed conversion (Remus et al., 1995; Augustine et al., 1997) noted in coccidia-challenged birds supplemented with natural betaine. Spreeuwenberg and coworkers (2007) noted an improvement

in feed efficiency of pigs challenged by subpar hygiene. Additionally, betaine supplementation in swine has been found to increase ileal dry matter, neutral detergent fiber and glycine digestibility (Ratriyanto et al., 2010). These authors also noted an increase in the bacterial metabolites D-lactic acid (in the ileum) and in fecal diaminopimelic acid as betaine level increased, this suggests that betaine may affect bacterial communities in different parts of the GI tract. A finding also suggested in the work of Eklund and coworkers (2006 a, b) in swine and Kettunen et al., 1999 in broilers. The ability of betaine to improve the efficiency of water balance control may be an important mechanism that allows a cell to “cope” with the conditions it is in.

Research with isolated tissues has demonstrated betaine’s role during osmotic stress as either ‘salt’ or heat stress. During hyperosmotic stress (environment is more concentrated in salt/solutes than the cell itself, thus causing water loss from the cell. One mechanism cells use to control water loss during a hyperosmotic stress is to increase electrolyte level within the cell. This increase in electrolytes (namely potassium) makes the cell more concentrated than its environment, so water is attracted back into the cell. However, the presence of betaine within the cell means that potassium level does not have to be increased in response to this type of stress. Indeed, work with piglet ileum noted that betaine supplemented piglet ileum had similar levels of potassium as tissue held at iso-osmotic conditions but piglet ileum exposed to hyperosmotic conditions in the absence of betaine had increased potassium levels (Tiihonen, personal communication). Additionally, isolated chicken duodenum (Kettunen et al., 2001a) and liver (Tiihonen, personal communication) could hold water more efficiently when betaine was present during hyperosmotic conditions such that water content was similar to tissue at isosmotic conditions (no hydration stress). Since accumulation of betaine involves less energy expenditure than electrolyte accumulation, the cell is left in a more efficient state energetically. In addition, betaine has no harmful interactions with enzymes (Burg, 1994; Petronini *et al.*, 1992; 1994), unlike electrolytes.

Macrophages, as part of the cellular immune response, help protect barrier tissues. As a result they are exposed to fluctuating osmotic conditions, work with mammalian macrophages reveals these cells accumulate betaine (eg. Zhang et al., 1996). Work by Klasing et al., 2002 noted that supplementation of betaine improved chemotaxis (migration in response to a chemical signal) and phagocytosis of macrophages in hyperosmotic and iso-osmotic conditions. Additionally, these authors noted increased numbers of leukocytes present on the villus and in the lamina propria of the duodenum.

Heat stress has also served as a model for the osmotic role of natural betaine. Gabler and coworkers (2013) noted that betaine mitigated heat stress-related changes in permeability as measured by ileal and cecal transepithelial electrical resistance and ileal fluorescein isothiocyanate-labelled dextran permeability in swine. Mooney and collaborators (1998) reported that broilers given natural betaine had higher levels of water retention than non-treated birds using cycling heat stress and/or coccidia-challenge as stressors.

Therefore, betaine, as an osmolyte, can influence water balance in cells, tissues and ultimately, the whole animal. The key is in the amount of betaine given. Research using lesion scores as an indicator of osmotic benefit has shown that about 500 g betaine/metric ton is needed to be able to see a response. Since the gastrointestinal system, liver and kidney have first exposure to betaine, to see much “spill over” of betaine from these tissues, at least 750 g betaine/ton must be supplied in the diet (Tiihonen, personal communication). Table 1 illustrates results from various heat stress trials with natural betaine supplementation.

Table 1. Responses noted to natural betaine under heat stress conditions.

Trial	Application	Parameter	Betaine	Control (P value)	Improvement %
USA.B.96.21	water	Water retention (ml)	95 ^a	83 ^b (0.05)	14.5
USA.B.06.44	water ¹	Survival (%)	93.8 ^{ab} /98 ^a	84.9 ^b (0.06)	10.5 14.2
USA.B.06.45	feed	FCR (g/g)	1.94 ^a	2.07 ^b (0.05)	6.3
University of Missouri, 1999	feed	FCR (g/g)	2.20	2.27 (0.32)	3.0
EGY.B.01.40	feed ¹	Corrected FCR (g/g)	1.93 ^{ab} /1.88 ^{ab} /1.81 ^a	2.01 ^b (0.05)	4-10
GRE.B.01.42 ²	feed	Corrected FCR (g/g)	1.73 ^a	1.84 ^b (0.05)	11
Thailand 1, 2000	water ¹	Mortality (% control)	48/61	100	39-52
MTR.B.THAI.06.03	water	FCR (g/g) Mortality (%)	1.84 3.72	1.95 5.28	5.6 29.5
MTR.B.ISR.01.02 ²	feed ¹	Corrected FCR (g/g)	1.75/1.72	1.77	1.1-2.8
NZ.B.13.55	feed	Wt corrected FCR (g/g) BMV (% LW)	1.55 21.5	1.66 (0.05) 19.3 (0.05)	7.1% 10%

¹ >1 Betaine treatment, ² reduced supplementary methionine and/or choline in Betaine treatment

Betaine's osmolyte role reduces the energy cost of cellular water balance as shown by Moeckel and coworkers (2002) wherein activity of ion pumps like Na/K ATPase and Ca²⁺ ATPase were reduced by 64% and 73%, respectively, in the presence of betaine. This can be real maintenance energy savings for the cell when ion pumps like Na⁺/K⁺ ATPase can require up to 2 ATP to ultimately move one molecule of potassium into a cell. Evidence in growing boars indicates that betaine decreases maintenance energy expenditure by 10% (Campbell et al., 1997) whereas a 5% reduction in maintenance energy was noted in market barrows (Schrama *et al.*, 2001). Since boars have a higher potential for lean tissue growth than barrows, it is possible that potential for lean growth also affects the maintenance energy expenditure on water balance given high water content of muscle.

This concept around lean potential and maintenance energy does give rise the prospect that the response to betaine supplementation in terms of carcass parameters (backfat thickness and lean percentage) could depend on whether the pigs are below or have reached their genetic capacity for protein accretion (Figure 7) compared to their energy intake. Pigs that are still in the linear portion of the curve could respond to an increased energy value of the diet with an increased protein deposition. The net result is less backfat and potentially leaner carcass. Conversely, pigs that have reached their genetic ceiling or have maximized energy intake relative to gain will not respond to betaine supplementation with increased protein deposition. Instead the extra energy available could be used to deposit more carcass fat/thicker backfat (eg response differences seen in the restricted versus *ab libitum* intake treatments in the work of Campbell et al., 1997, Casarin et al., 1997, Matthews et al., 1997; Suster et al., 2004). This would help explain the contradictory findings in the scientific literature on carcass response to betaine where some saw a benefit (Fernandez-Figares et al., 2002; Siljander-Rasi et al., 2003; Dunshea et al., 2007) and others did not (Fernandez-Figares et al., 2008) on carcass.

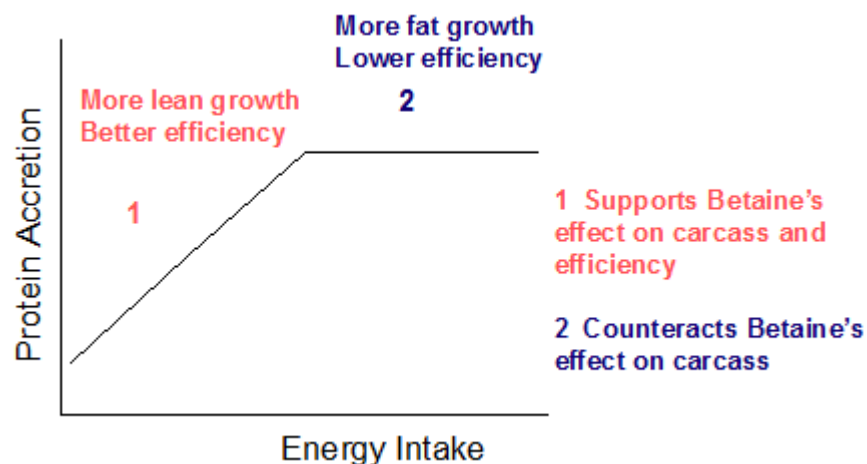


Figure 7. Relationship between energy intake and rate of protein deposition.

Evidence of this phenomena can be seen in the work of Casarin and coworkers (1997) where animal intake was either at ad libitum or at 80% of ad libitum in the presence/absence of 1 kg natural betaine/metric ton (Table 2). Overall, feed conversion was improved by betaine supplementation in both barrows and gilts fed on a restricted basis. Pigs fed betaine had less backfat at all levels of intake: 23% improvement for pigs fed *ad libitum* and 28% for the feed restricted pigs. The effect of betaine supplementation on percent lean was more pronounced in gilts than barrows. The results of this experiment suggest that betaine supplementation would be most effective in improving rate of gain under conditions where feed intake is below the pig's potential.

Table 2. Interactive effects of betaine and feed intake level on growth performance of growing-finishing pigs

Criterion	Barrows				Gilts			
	Ad libitum		80% of Ad libitum		Ad libitum		80% of Ad libitum	
	Control	Betaine (1kg/t)	Control	Betaine (1kg/t)	Control	Betaine (1kg/t)	Control	Betaine (1kg/t)
Grow-finish performance								
ADG, kg/d	0.932 ^a	0.929 ^a	0.798 ^b	0.813 ^b	0.830 ^b	0.803 ^b	0.724 ^c	0.809 ^b
ADFI, kg/d	2.385 ^a	2.349 ^a	1.767 ^d	1.733 ^d	2.198 ^b	2.008 ^c	1.678 ^d	1.733 ^d
Feed:gain	2.559 ^d	2.528 ^d	2.214 ^b	2.131 ^a	2.648 ^d	2.500 ^d	2.317 ^c	2.142 ^a
Carcass								
Dressing %	83.4 ^a	82.7 ^a	80.7 ^b	80.1 ^b	83.0 ^a	82.7 ^a	80.7 ^b	80.2 ^b
Backfat, mm	14.2 ^d	11.2 ^c	12.0 ^c	10.5 ^b	13.0 ^d	9.7 ^a	13.0 ^d	7.5 ^a
Lean meat, %	51.0 ^d	50.6 ^d	52.2 ^c	52.3 ^c	51.7 ^d	54.4 ^b	53.0 ^b	55.1 ^a

a,b,c,d Means within a row with different superscripts are significantly different ($P < 0.05$)

The major benefit of using betaine in swine operations is with the "energy boosting" effect of betaine. In the case of pigs with submaximal energy intake, betaine addition can result in improvements in feed efficiency in the range of 6.5% (Figure 8). If energy intake is sufficient to support maximal lean growth, such as is the case when high fat levels (4-7%) are included in the diet, the utilization of betaine results in cost savings by allowing for the removal of part of that fat without affecting the performance of the pigs.

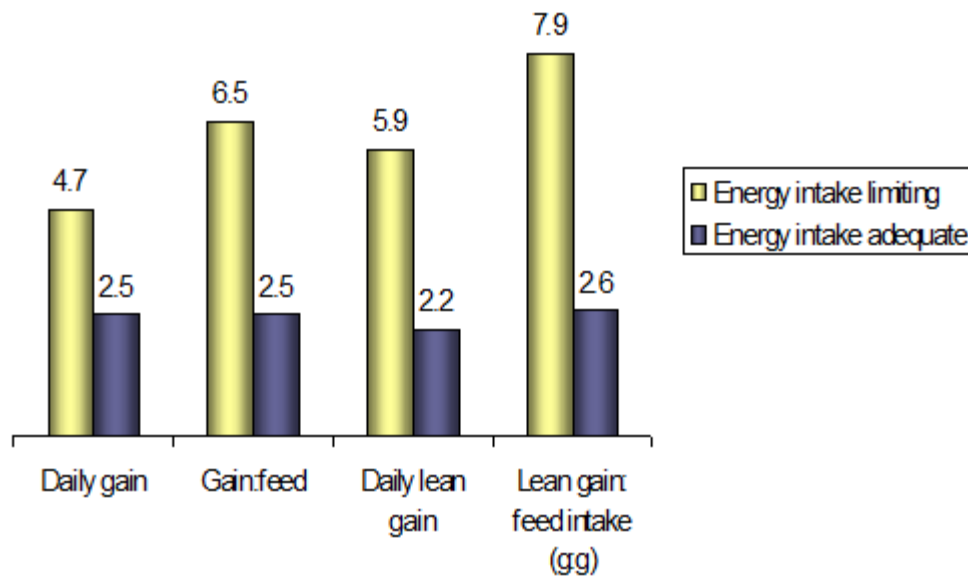


Figure 8. Response (%) to betaine is magnified when energy intake limits daily lean gain. Mean of trials: References = Betafin.S.GER.94.08; Betafin.S.GER.94.09 Betafin.S.USA.96.24; Betafin.S.MEX.96.21; Betafin.S.USA.98.32; Betafin.S.USA.98.33

Meat birds have also shown carcass responses to natural betaine supplementation although these often are noted as increased white meat yield in broilers (Virtanen and Rosi, 1995) and turkeys (Kalbfleisch *et al.*, 2001; Noll *et al.*, 2002). Changes in abdominal fat pad have also been reported (Virtanen and Rosi, 1995). Evidence has emerged that betaine may affect intramuscular fat accumulation in swine (Albuquerque *et al.*, 2017), something that may influence sensory/taste aspects of pork to the consumer.

Benefits of betaine supplementation extend beyond the growing animal. Cabezon and coworkers (2016) noted higher sperm concentration in ejaculated from mature boars exposed to summer heat stress and supplemented with 0.63% betaine. Evidence from sow trials where betaine has been supplemented have noted effects on parameters such as litter weight (Ramis *et al.*, 2011), rebreeding interval (Ramis *et al.*, 2011; Greiner *et al.*, 2014; Cabezon *et al.*, 2016) and number borne in the next litter (Campbell *et al.*, 1997, Ramis *et al.*, 2001, van Wettere *et al.*, 2008). The work of Campbell *et al.*, 1997 and van Wettere *et al.*, 2012 both noted higher response to betaine on subsequent litter size in higher parity sows. Additionally, work ran in hot summer weather noted a tendency toward higher number of piglets born (van Wettere *et al.*, 2012; Greiner *et al.*, 2014) and born live (van Wettere *et al.*, 2012) in the next parity as well as shorter breeding interval (Greiner *et al.*, 2014). Figure 9 notes an average of 1 additional piglet born live to sows given natural betaine in either their lactation or gestation diets.

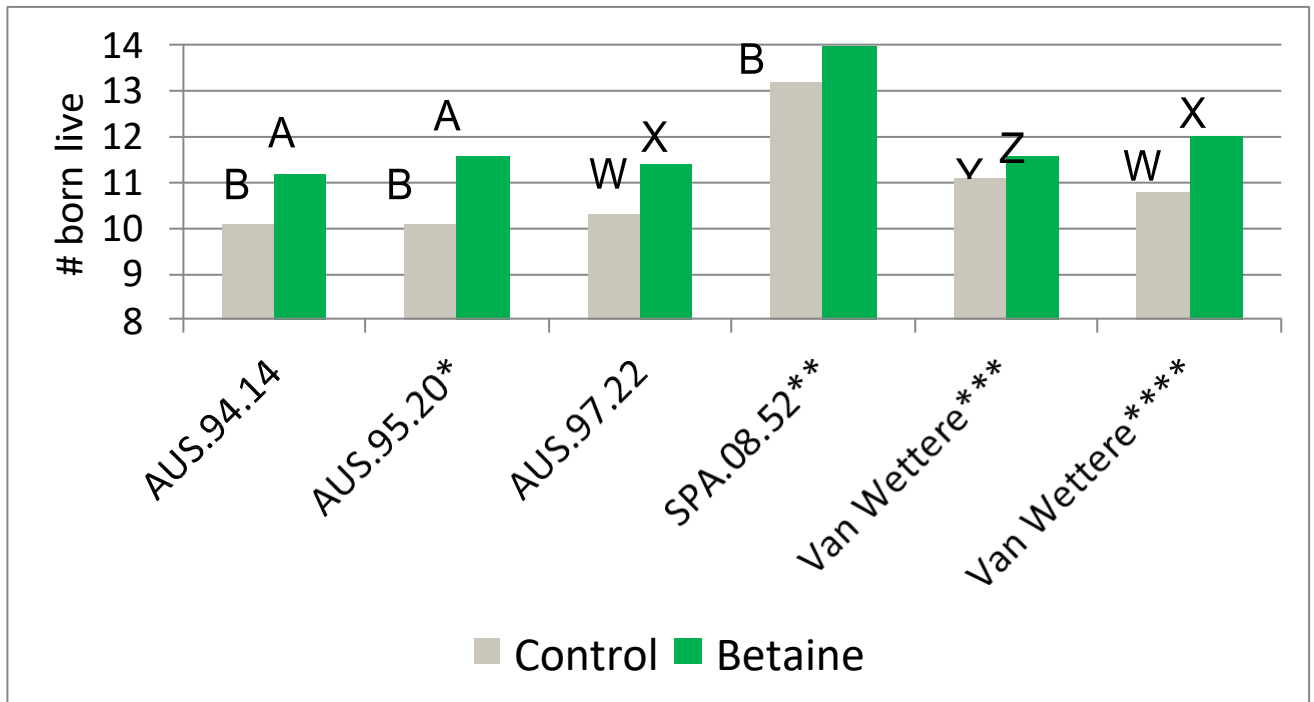


Figure 9. Influence of 0.2% natural betaine on litter size as born live.

*Campbell et al., 1997, **Ramis et al., 2011, ** Van Wettère et al., 2008 - average all parities (gestation); *** Van Wettère et al., 2008 - average parities 3 to 7 (gestation)

Y, Z: means without a common letter within a trial differ at $P < 0.08$

W, X: means without a common letter within a trial differ at $P < 0.06$

A, B: means with a common letter within a trial differ at $P < 0.05$

Since betaine is used both as a methyl donor and osmolyte in livestock diets, optimizing dietary methyl donors can be one way to offset cost of betaine entry into diets. Typical betaine additions (100% basis) range from 0.0375 to 0.1% of the diet for meat birds/layers and from 0.1 to 0.2% for growing or reproductive swine. As betaine is an osmolyte from moment of consumption, it is this role that will be observed first at the gastrointestinal level and later in other tissues depending on level supplemented and liver methyl group demand.

Final Thoughts

The dual functions of betaine in the body allow it to be used a variety of ways, depending on what is the desired response is. Use of betaine as an osmolyte helps protect cells against water loss, but it also can be used to benefit efficiency of livestock during periods of stress. As a methyl donor, betaine promotes regeneration of methionine in the liver, which allows optimized dietary addition of choline and/or methionine in livestock diets. Overall, the activities of betaine in livestock nutrition make it a valuable tool in optimizing performance.

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L-carnitine for Sows: 25 Years of Research-Developed Applications

La L-carnitine chez les truies : 25 années d'applications nées de la recherche

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Abstract

L-carnitine is a vitamin-like nutrient that is essential for proper fat and energy metabolism, and also plays a role in protein and carbohydrate metabolism in the body. Because of its impact on metabolic processes, over 25 years of research has demonstrated that L-carnitine supplementation in sow gestation and lactation diets results in improved performance. Much of the early research focused attention on improving litter size. Especially interesting with the modern hyperprolific sow is its ability to improve piglet birth weight and reduce the number of non-viable pigs born per litter. This impact on fetal development has also been shown to positively influence fetal muscle development resulting in greater carcass lean in offspring from sows fed diets with L-carnitine. Improvements in litter weight gain during suckling have been reported due to increased milk production and enhanced piglet suckling activity. Sow body weight and backfat gain have also been reported during gestation because of more efficient feed utilization. New research is suggesting even more potential applications for L-carnitine in sow diets. Recent data suggests that L-carnitine may shorten the time of parturition, reduce protein catabolism, influence gut barrier function and microbiota, and influence muscle maturation of low-birth weight pigs if supplemented in a milk replacer. Overall, there is a long history of L-carnitine use in sow diets and recent research is suggesting new applications that could be beneficial for the modern hyperprolific sow.

Résumé

La L-carnitine est un élément nutritif de type vitamine qui est essentiel pour le métabolisme des graisses et de l'énergie et qui joue aussi un rôle dans le métabolisme corporel des protéines et des hydrates de carbone. Plus de 25 années de recherche sur ce composé important dans les processus métaboliques ont montré que l'ajout de L-carnitine dans les rations de gestation et de lactation chez la truie entraîne une amélioration de la performance. Les premières études ont en bonne partie porté sur l'augmentation de la taille des portées. Chez la truie hyperprolifère moderne, l'aptitude de la L-carnitine à améliorer le poids à la naissance des porcelets et à réduire le nombre de porcelets non viables nés par portée est particulièrement intéressant. On a aussi montré que cet effet sur le développement fœtal a une influence positive sur le développement musculaire du fœtus qui se traduit par un meilleur rendement en maigre de la carcasse des porcelets nés de mères nourries avec des rations additionnées de L-carnitine. Des améliorations

notées au chapitre du gain de poids de la portée pendant l'allaitement ont été associées à l'augmentation de la production laitière et à la stimulation du réflexe de tétée chez les porcelets. Des gains de poids corporel et de gras dorsal chez la truie ont aussi été associés à une utilisation plus efficace des aliments durant la gestation. De nouvelles études laissent entrevoir encore davantage d'applications potentielles pour la L-carnitine. Selon des données récentes, la L-carnitine pourrait raccourcir le temps de parturition, réduire le catabolisme des protéines, influencer la fonction de la barrière intestinale et le microbiote et intervenir sur la maturation des muscles des porcelets de faible poids à la naissance quand on l'ajoute au lactoreemplaceur. En résumé, l'utilisation de la L-carnitine dans l'alimentation des truies remonte à loin, et des travaux récents suggèrent de nouvelles applications qui pourraient être bénéfiques pour la truie hyperprolifique moderne.

Introduction

L-carnitine is often referred to as a conditionally essential nutrient because while the body does produce L-carnitine, additional supplementation yields benefits in metabolic efficiency as well as improvements in performance. L-carnitine is chemically defined as γ -trimethylamino- β -hydroxybutyrate. It is present in two main forms (L- and D-carnitine) but only the L-isomer of carnitine is found in nature and biologically active. In the body, L-carnitine is synthesized starting from the amino acids methionine and lysine (Hoppel and Davis, 1986). L-carnitine is mostly known for the role it plays in energy metabolism. Different well documented studies conducted by various Universities and research organizations around the world have proven this function in both humans and animals. When it comes to energy metabolism, L-carnitine is primarily involved in shuttling fatty acids across the inner-mitochondrial membrane where they are metabolized and converted into energy. In addition to its role in fatty acid metabolism, L-carnitine is also known to impact normal carbohydrate and protein utilization in the body. In particular, L-carnitine supports the cells in maintaining a sufficient level of free CoA by temporarily binding and storing activated acyl-moieties. This specific buffer function supports an optimum energy metabolism in the mitochondria (Tein, 2003). Raising the L-carnitine concentration in the cell plasma enhances the body's ability to obtain energy from fatty acids and carbohydrates, reduces the catabolism of amino acids and fosters protein accretion (Owen et al., 2001b). Because of the role L-carnitine play in optimizing energy metabolism, supplementation in sow diets has resulted in improvements in production for both the sow and her offspring.

Proven benefits of L-carnitine supplementation for sows

Application of L-carnitine as a specialty feed ingredient used for influencing sow productivity has a long track record. The first report of supplementing L-carnitine to sows was in 1993 when Fremaut et al. showed that supplementing 250 mg/d resulted in increased litter size and suckling growth rate. Since that report 25 years ago, other research has demonstrated benefits of L-carnitine supplementation in gestation and lactation diets as reviewed below.

L-carnitine increased number of pigs born alive: Much of the original research conducted with L-carnitine was focused on its ability to improve the number of piglets born and born alive. A number of researchers from different geographical locations, feeding different basal diet

formulations, to different genetic lines of pigs reported improvements in litter size (Musser et al., 1999; Ramanau et al., 2002; Ramanau et al., 2004; Ramanau et al., 2008) and reductions in number of stillborns (Eder et al., 2001; Ramanau et al., 2002; Birkenfeld et al., 2006a). While many of these trials have also reported increases in number of pigs weaned from L-carnitine supplementation, the genetic advancements in productivity of modern hyperprolific sows makes these benefits less interesting than what they were when the trials were conducted. However, the ability to influence litter size without negatively impacting birth weight, and in many cases concomitantly increasing birth weight, is what is gaining significant interest today.

L-carnitine improves fetal development: A number of studies have reported that when L-carnitine is included in gestation diets, the piglet birth weights in the subsequent litter will be increased (Musser et al., 1999; Eder et al., 2001; Ramanau et al., 2002; Eder et al., 2003; Birkenfeld et al., 2006a; Ramanau et al., 2008) as well as mid-gestation fetal weights increased with L-carnitine supplementation (Brown et al., 2008). Even when the number of piglets born alive is increased, the piglet birth weight is maintained or greater when L-carnitine is included in the diets (Ramanau et al., 2004; Ramanau et al., 2008), a relationship which is normally inversely related. These findings would suggest that L-carnitine will elicit beneficial effects on fetal growth and development.

Other research has demonstrated that L-carnitine supplementation will reduce the number of non-viable (less than 800 grams) piglets born (Eder et al., 2001; Ramanau et al., 2002; Eder et al., 2003). Ramanau et al., 2008 first showed that the distribution of piglet birth weights shifted such that there were an increased number of heavier birth weight pigs and fewer lightweight pigs. Recently, O'Driscoll et al. (2017) observed the same response with a higher proportion of pigs with heavier birth weights compared to those with light birth weights.

Other research has reported that L-carnitine fed in gestation diets will elicit increased muscle development of the offspring. Musser et al. (2001) reported that in two studies, piglets obtained from sows fed diets containing L-carnitine during gestation had 27.8% and 6.5% more muscle fibers compared to piglets obtained from sows fed diets without L-carnitine. In a third study, Musser et al. (2000) confirmed that L-carnitine enhanced muscle fiber development of the offspring and observed that the benefit was maintained at slaughter as piglets from sows fed diets with L-carnitine had greater loin depth (59.4 vs 57.0 mm) and lean percent (55.1 vs 54.5%).

The exact mode of action for L-carnitine's ability to improve fetal growth and development is unknown; however, it is most likely related to its ability to influence energy metabolism (Owen et al., 2001), increase maternal IGF-1 concentrations (Musser et al., 1999), or influence maternal leptin concentrations (Woodworth et al., 2004). In addition, Waylan (2005) recently observed that maternal L-carnitine influenced the expression of fetal mRNA for IGF-1, IGF-2, IGFBP-3, and myogenin, resulting in increased proliferation of embryonic myocytes. The increased proliferation observed by Waylan (2005) could be the reason that increased muscle expression was observed by Musser et al. (1999, 2000). Additionally, Brown et al. (2007, 2008) determined that supplementing L-carnitine to sows influenced expression of key hormonal systems that support enhanced fetal development. These results suggest that L-carnitine fed during gestation will elicit a beneficial impact on fetal growth and development that can be observed in increased birth weights, reduced stillborns, and increased muscle development.

L-carnitine supplementation in lactation diets improves litter performance: Past research has shown that when sows are supplemented with L-carnitine during lactation, the piglets grew faster compared to the piglets of control sows (Eder et al., 2001; Ramanau et al., 2002). Other research (Ramanau et al., 2004) confirmed these observations and suggested that the improved growth performance is a reflection of increased milk production. In their experiment, Ramanau et al. (2004) allotted sows to diets with or without supplemental L-carnitine and sows were fed their respective diets for two parities. Sows fed diets containing L-carnitine had larger litters and greater litter weights. The individual birth weights of the piglets was not different between treatments; however, the piglets from sows fed diets with L-carnitine were heavier at weaning, reflecting their enhanced growth rate during suckling. These researchers also determined milk yield at day 11 and 18 of lactation and observed that when sows were fed diets containing L-carnitine, milk yield increased. The increased milk yield resulted in an increase in the amount of nutrients secreted in the milk each day. Averaged over both parities, there was 11.5% more fat, 20.4% more protein, and 21.9% more lactose secreted on a daily basis when sows were fed diets with L-carnitine. In addition the L-carnitine concentration of the milk was increased 35% when L-carnitine was supplemented to the sows. The fact that sows fed diets containing L-carnitine also had increased daily feed intake during lactation may explain part of the increased milk output that was observed and may explain why not all studies show changes in milk composition from L-carnitine supplementation (Birkenfeld et al., 2006a).

In addition to the above, another reason that suckling weight gain is increased when sows are supplemented with L-carnitine is that piglet activity is improved. Data shows that piglets that are nursing sows supplemented with L-carnitine will nurse for longer duration per suckling event and the total suckling time per day will be increased (Birkenfeld et al., 2006b). This could be the result of enhanced carnitine status of the newborn piglets (Birkenfeld et al., 2006a; Xi et al., 2008) allowing for more efficient use of fat in the colostrum and milk resulting in enhanced growth and a greater demand for nutrients to support the extra growth.

L-carnitine influences maternal body weight gain during gestation: One of the other benefits of L-carnitine supplementation during gestation is its ability to improve the efficiency of energy utilization such that sows will have more weight and backfat gain on the same amount of feed. A number of studies have demonstrated this in mixed parity sows (Musser et al., 1999; Eder et al., 2001; Birkenfeld et al., 2006a) and gilts (Brown et al., 2007). These benefits result in more body weight recovery after lactation if producers feed the same amount of feed, or reduced daily feed needs if producers feed to a targeted body condition basis.

Recent discoveries and new applications of L-carnitine for sows

While the history of L-carnitine supplementation in sow diets is extensive, it is the new discoveries that are uncovering additional benefits that may result in its greatest applications in commercial swine herds around the world. Considering the high level of productivity of modern genotypes as well as the challenges being faced by the global swine industry today, below are highlights of some of the most recent research that could transfer into additional justification for L-carnitine supplementation to sows.

L-carnitine Shortens Parturition Time: The modern hyperprolific sows with litter sizes of 16+ can have a farrowing process lasting 6-8 hrs or higher. This can lead to increased stillborn rates and some suggest the increase in sow prolapse our industry is experiencing is due to the extended time of farrowing. Kotowski et al. (2006) conducted a study where they fed 250 mg/d of L-carnitine in gestation and 350 mg/d in lactation. While the L-carnitine supplementation increased piglet birth weight and piglet weight gain during suckling similar to other trials, this paper also demonstrated that the farrowing process was quicker with L-carnitine supplemented sows even though litter size was similar. In addition, puerperal toxemia was lower in the L-carnitine supplemented sows compared to the non-supplemented sows. L-carnitine may be eliciting these benefits because of its ability to improve energy metabolism, especially in the smooth muscles like those associated with the uterus. This is the only trial that can be found that shows the influence of L-carnitine on parturition time, but additional research should be conducted to confirm these results as parturition time will increase as litter size continues to increase.

L-carnitine Reduces Protein Catabolism: Keller et al. (2012) showed that when pigs were fed diets supplemented with L-carnitine, genes associated with the break-down of protein were down regulated. This could help explain some of the previous research with swine that showed protein accretion and final carcass muscling of finishing pigs was increased with L-carnitine supplementation (Owen et al., 2001a). In addition, this could be a major finding for sows in that it could support reduced protein catabolism during lactation which could mean a less severe “second-parity slump” and increased retention in the herd.

Maternal L-carnitine Influences Offspring Intestinal Barrier Function: Wei et al. (2016) supplemented L-carnitine in gestation (100 mg/kg) and lactation (200 mg/kg) and observed increased antioxidant capacity in the offspring jejunal mucosa, decreased concentration of cytokines in the mucosa (i.e. IL-1, TNF- α among others), increased expression of tight junction proteins, and changes in offspring gut bacteria (such as increased *Lactobacillus*). These findings illustrate the ability of maternal nutrition to influence offspring GIT health. In particular, they show the ability of maternal L-carnitine to potentially elicit a healthier GI tract which could better prepare offspring for the stresses associated with weaning.

L-carnitine in Milk Replacers: Madsen et al. (2018) demonstrated that if L-carnitine was supplemented in a milk replacer to low-birth weight piglets from d 7 of life until weaning at d 28, muscle maturation was improved. In addition, growth rate during the first week of supplementation tended to be greater with L-carnitine supplementation. These benefits could offer a solution for improving performance of the increasing numbers of low-birth weight pigs produced from today’s hyperprolific sows.

Conclusion

Over 25 years of research with L-Carnitine in gestating and lactating sow diets has yielded benefits in maternal and offspring performance. Recent research with this vitamin-like compound is uncovering new applications that still suggest its application for the modern hyperprolific sow.

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Novel Products/Technologies Affecting Rumen Fermentation and Ruminant Microbiome

Produits et technologies novateurs intervenant sur la fermentation ruminale et le microbiome des ruminants

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Abstract

In monogastric animals, Tasco[®], a solar dried, high quality macroalga product made from *Ascophyllum nodosum* exhibits potent prebiotic activity that improves Gastro-intestinal (GI) tract health. This results in productivity and health improvements. Prebiotics are feed constituents that promote the growth of beneficial bacteria in the large intestine over the growth of pathogens and thus improve the GI health of the animal. In ruminant animal we see similar improvements in production, health and stress resistance but ruminants also have a second site of microbial fermentation located proximally in the GI tract in a specialized organ called the rumen. When Tasco[®] is fed to ruminants, total rumen bacteria counts are decreased but this decrease is accounted wholly by decreases in pathogenic bacteria. Also decreased were populations of methanogenic bacteria (archaea) associated with the loss of carbon substrate as methane, a greenhouse gas. Populations of protozoa, implicated in cellulose fiber degradation, were significantly increased. Overall these changes to the rumen microbiota explain increased digestive efficiency of the ration as well as improved productivity and health status of the animal when fed Tasco[®]. In conclusion, in ruminants, Tasco[®] acts not only like a potent prebiotic but also as a potent rumen modifier, biasing rumen physiology to the benefit of the animal in terms of production and health.

Résumé

Chez les monogastriques, Tasco[®], un produit de macroalgue de haute qualité à base de *Ascophyllum nodosum* séché au soleil, exerce une puissante activité prébiotique qui améliore la santé du tube digestif. Cela se traduit par un gain de productivité et une meilleure santé. Les prébiotiques sont des composants alimentaires qui favorisent la croissance des bactéries bénéfiques dans le gros intestin, au détriment des agents pathogènes, et qui, par conséquent, améliorent la santé du tube digestif de l'animal. Des effets similaires sur la production, la santé et la résistance au stress sont observés chez les ruminants, mais ces animaux disposent en plus d'un second site de fermentation microbienne adjacent au tube digestif, dans un organe spécialisé appelé le rumen. L'administration de Tasco[®] aux ruminants entraîne une diminution des comptes bactériens totaux dans le rumen, mais cette baisse est due uniquement à la réduction des bactéries pathogènes. De plus, une diminution des bactéries méthanogènes (archées) a été associée à la perte de substrat de carbone sous forme de méthane, un gaz à effet de serre. Les populations de

protozoaires, qui interviennent dans la dégradation de la cellulose, ont augmenté significativement. Globalement, ces modifications du microbiote ruminal expliquent la digestion plus efficace ainsi que les gains de productivité et de santé observés chez les animaux qui ont reçu Tasco®. En conclusion, chez les ruminants, Tasco® agit non seulement comme un puissant probiotique, mais aussi comme un puissant modificateur du rumen, influençant la physiologie ruminale en faveur de la productivité et de la santé de l'animal.

Introduction

Seaweeds have long been used in animal feeding programs. These references date back as far as 2000 thousand years to the days of the Roman empire (Evans and Critchley, 2013). Associated with the supplementation of seaweed in animal rations there have long been anecdotal benefits cited for improved productivity and health. However, it is only recently that the science has caught up with understanding the role of the various constituents contained within the seaweed when consumed by animals on GI tract health and nutrition.

Impressed with the presence of large unused natural beds of the resource and high growth rates of seaweed, early scientists explored the use of supplementation of this biomass in animal rations. These early studies generally used high rates of incorporation of seaweed as replacements for expensive grains and other conventional energy sources. Results of these trials were generally disappointing and recommendations of professional nutritionists at the time were that seaweeds were not recommended in animal rations (Morrison, 1974). Nevertheless, the use of seaweed supplementation in some animal production sectors persisted based on what the producers observed, such as in the natural and organic dairy production sectors.

With the knowledge we have today, these early feeding trial results are hardly surprising. Seaweed polysaccharides vary widely in composition between species, but generally contain complex, branched molecules, virtually undigestible by the GI tract enzyme systems in higher animals (Raposo et al., 2016). Therefore, when incorporated into animal rations at high levels they reduce the metabolizable energy density of the feed and result in reduced growth performance. Although undigestible fiber (i.e. crude fiber) has long been known to have a role in maintaining gut health it was not until the publication by Gibson and Roberfroid (1995) that a special type of soluble fiber was reported to be a key component in promoting GI tract health. Special fiber sources, undigestible by higher animal enzyme systems but selectively fermentable by beneficial microorganisms within the GI tract of animals have been shown to significantly improve GI tract health. These special fibers have been termed “prebiotics” (Roberfroid, 2007). In whole animal trials, when fed robust prebiotic ration for their entire life, rats were found to have lifespans, extended by 30% compared to Control animal (Rozan et al., 2008). This shows the importance of maintaining a high level of GI tract health on general health, productivity and longevity.

Like terrestrial plants, marine plants vary widely in composition by species, location, nutrient availability and environment. Of all seaweed species the most scientifically studied has been a seaweed species commercially harvested from the north Atlantic Ocean region, called *Ascophyllum nodosum*. And, of all the commercially harvested seaweed biomass, the most

researched has been the branded product made from this species called Tasco[®]. Tasco[®] is a very high-quality seaweed meal, produced from freshly harvested material of the macroalga, *Ascophyllum nodosum* (i.e. not significantly contaminated with other seaweed species), and sun-dried by a low temperature process to preserve all of the bioactivity of the compounds contained therein.

Feeding Tasco[®] to Animals

Early research with Tasco[®] had shown that it contained at least three groups of bioactive constituents that appeared to be of importance for animal feed supplementation. As determined by proximate analysis, Tasco[®] contains 52% DM carbohydrate content (CHO), characterized by a suite of complex polysaccharides. Alginate, a complex, linear polysaccharide with many cross-linking bonds accounts for 25% dry matter (DM). A complex, branched, highly sulfated polysaccharide named “fucose containing polymer” or FCP, is found at about 15% DM, often referred to by its trivial name as “fucoidan”. Lesser amounts of polysaccharides include laminarin, a β 1,3; β 1,6 polysaccharide, is found at about 3% DM and is similar in structure to polysaccharides found from bacterial or fungal sources. In addition, mannitol, a sugar-alcohol, at about 5% DM is the simplest of CHO's present with the remainder of 4% DM made up of other intermediate CHOs of complex and variable structure. It is significant, however, to note that Tasco[®] does not contain any simple, readily fermentable sugars, nor any linear, highly digestible starches like terrestrial plants contain that are readily hydrolysable energy sources.

In addition to the CHO content, Tasco[®] also contains significant quantities of phlorotannins at 5 to 7% DM. Phlorotannins are large, complex molecules made up of smaller building blocks of a highly polyphenolic compound called phloroglucinol. Phlorotannins have only recently been studied in animal feeding studies and results conclude that feeding phlorotannins, *in vivo*, result in highly selective action against endogenous pathogenic micro-organisms without any of the anti-nutritional effects seen in terrestrial plant, condensed tannin feeding trials (Nagayama et al. 2002). Interestingly, digestion of phlorotannins enhances the growth inhibitory effect, at least as determined by *in vitro* methodology (Corona et al., 2017)

The last major constituent or bioactive component that Tasco[®] has been found to contain is its antioxidant action. This action is thought to be mediated by a suite of compounds including the polyphenolic phlorotannins described previously, as well as a suite of flavonoids compounds and antioxidant pigments such as fucoxanthin (Holt and Kraan, 2011). These antioxidant compounds result in a very high capacity to reduce reactive oxygen species (ROS), a damaging by-product of cellular activity and energy metabolism. During digestion and passage through the GI tract phlorotannin products are metabolized and taken up by tissues where it effectively mitigates the ROS damage *in situ* as shown by *in vivo* trials using *C. elegans* and neural node accumulation of ROS. Reduction in ROS exposure greatly enhances stress resistance (Kandasamy et al., 2011).

When Tasco[®] is fed to monogastric animals in commercial animal agriculture, improvements in production related benefits as well as improvements in health status can be observed. These improvements include stress resistance, immune competency, productivity and growth rates (Allen et al., 2001; Evans and Critchley, 2013). Results from poultry trials even show that

Tasco[®] may be a component of a production system that can replace sub-therapeutic use of antibiotics and can achieve growth rates similar to antibiotic treatment (Wiseman, 2012).

Ruminant Animals

When Tasco[®] is fed to ruminant animals, productivity and health status improvements similar to that seen in monogastric animals are observed. Dairy cows fed Tasco[®] increased milk production from 2 pounds, 4 pounds or up to 6 pounds per animal per day (Evans, 2008). Also observed are increases in growth performance or meat quality (Braden et al., 2007), reductions in somatic cell counts in milk cows (Kellogg et al., 2006) and heat stress resistance (Williams et al., 2009).

Ruminant animals, however, have a much more complicated digestive tract than monogastric animals. Instead of only one major site of microbial fermentation in monogastric animals, the large intestine at the distal end of the GI tract, ruminants have two sites. In ruminants, located proximally in the digestive tract, even before the true stomach where acidification occurs, is located the rumen. In this organ, an active, diverse microbial community is promoted in a special organ where active fermentation occurs. Volatile fatty acids (VFAs), acetate, propionate and butyrate are released and taken up directly by the host animal where they are utilized directly in energy production or to synthesize carbon containing metabolites. Protein is derived from the microbial biomass when it passes through into the regular stomach and small intestine followed by the large intestine where another population of microbial organisms ferment the residual material, just like in the monogastric animal.

The essential question is, however, does Tasco[®] have effect on fermentation in the rumen as well as large intestine and if so, how does it affect fermentation patterns? Feed additives that affect the rumen are termed rumen modifiers. Early research with Tasco[®] utilizing *in vitro* fermentation protocols, in efforts to simulate rumen function indicated that the phlorotannins, in particular, appeared to reduce fermentation gas production suggesting potential negative effects on whole GI tract digestion and energy recovery (Wang et al., 2007). But clearly, as cited earlier, when ruminants are fed Tasco[®], productivity is improved. Therefore, either Tasco[®] does not have effects in the rumen and all the improvement is due to enhanced fermentation in the large intestine, or Tasco[®] exerts some effects in rumen in addition to the large intestine. Phlorotannins from the seaweeds similar to that from which Tasco[®] is made have been used medically to delay free sugar release and thus reduce peak blood glucose levels by interfering with hydrolysis of starches from the digesta (Kellogg et al., 2014). If this action is widespread in the rumen when Tasco[®] is fed to ruminating animals, Tasco[®] could actually be expected to have detrimental effects on nutrient release and availability.

Tasco[®] Action in the Rumen

To investigate this question, Zhou et al., (2018) administered Tasco[®] to ruminants, utilizing sheep as the model animal. Rumen VFAs were measured as well as activity through metabolic pathways by use of molecular biology tools. Rumen microbiota populations were also measured

including protozoa, beneficial bacteria, pathogenic bacteria as well as archaea. Results did not show negative effects on key metabolic indicators that relate to effects on rumen fermentation and nutrient availability as predicted by *in vitro* trials cited earlier.

When samples of rumen contents were collected, the DNA was extracted and 16S rRNA was sequenced to study the ecology of bacterial and archaeal communities using QIIME molecular biology techniques. Results show that Tasco[®] treatment resulted in a reduction of total bacterial populations in the rumen (Figure 1). However, when the relative abundance of bacteroidetes, firmicutes and proteobacteria, all considered to be beneficial bacteria, were measured, no differences were found between the Control and Tasco[®] treated groups.

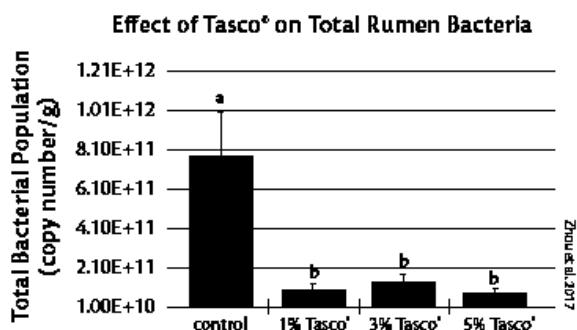


Figure 1. Tasco[®] treatment results in a decrease in total rumen bacteria populations.

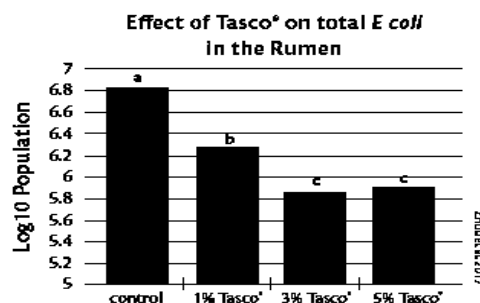


Figure 2. Tasco[®] treatment also reduces populations of pathogenic or harmful bacteria in the rumen.

When levels of pathogenic bacteria were analysed, decreases in relative abundance were associated with increasing Tasco[®] administration. Figure 2 shows a decrease in *E. coli* bacteria, a group typically representing pathogenic bacterial populations. Pathogenic bacteria within the digestive tract reduce GI tract health, waste nutrients and potentially provide for a disease outbreak situation whenever the animal becomes stressed.

More surprising, however, was the finding that Tasco[®] treatment significantly reduced archaea populations (Figure 3). Archaea are primitive, simple bacteria that have the unique ability to cheat metabolically by gaining energy directly from carbohydrates by releasing methane gas. Not only does this release of methane gas represent a waste of nutrients that otherwise would be converted to VFA's that the host animal can use for synthesis of other carbon compounds or energy liberation but that this methane is also a potent greenhouse gas.

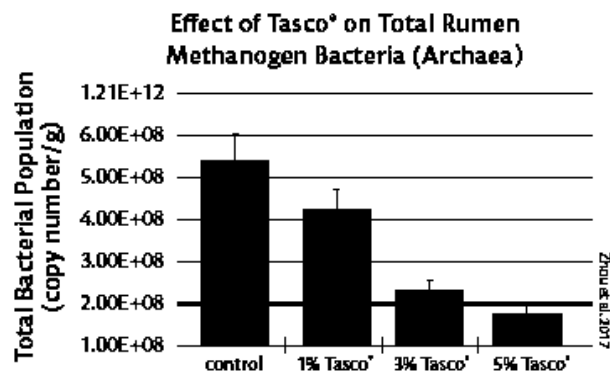


Figure 3. Tasco[®] has special effects on reducing archaea bacteria that waste rumen nutrients as methane gas.

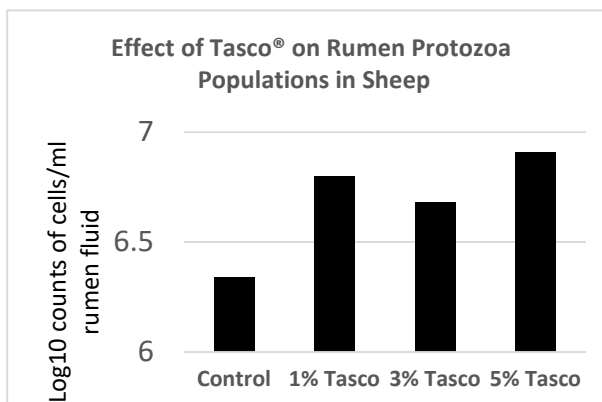


Figure 4. Tasco[®] treatment increases the population of rumen protozoa.

When concentrations of VFA's were analysed along with functional pathways of these metabolic products, results suggest that butanoate metabolic pathways were upregulated in Tasco[®] treated animals. These results suggest that the potential for fatty acid biosynthesis and fatty acid metabolism was increased in Tasco[®] treated animals. In fact, with Tasco[®] treatment, 14 metabolic pathways associated with VFA uptake were enriched and only three were suppressed. These changes in VFA metabolism are often associated with increased GI tract health.

In addition, Tasco[®] treatments were found to result in increases in rumen protozoa populations (Figure 4). Protozoa are normally associated with increased forage fiber digestion. They are implicated in the release of more nutrients and energy by their tunneling activity that exposes a larger surface area to hydrolytic bacteria.

Conclusions

Tasco[®] treatment alters the rumen microbiome in ways similar to the effects it has on the large intestine in monogastric animals. Tasco[®] treatment enhances beneficial bacterial populations while at the same time reduces pathogenic bacteria. The end result for the host animal is an increase in rumen/GI tract health. The potential decrease in methane release from the archaea bacterial populations could partly account for the apparent increase in feed ration digestibility and improved productivity of animals fed Tasco[®] as shown in previous trials examining performance characteristics of Tasco[®] treatment (Leupp et al., 2005). This is also potentially increased by the actions of the protozoa that result in higher fiber digestibility. The improved VFA metabolism is associated with improved fatty acid synthesis in ruminant animals and may explain the improved carcass grading index and improved intramuscular (marbling) fat deposition seen in previous studies (Braden et al., 2007).

In summary, this study has shown that Tasco[®] treatment alters the microbiome populations in the rumen in ways that significantly improve the metabolic consequences of rumen digestion. This

results in improved health, stress tolerance and increased feed digestibility. Tasco® appears to be a potent rumen modifier that has the potential to significantly improve ruminant productivity.

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A Current Understanding of DCAD – an Update on Reducing Hypocalcemia

État actuel des connaissances sur la DACA – une mise à jour sur les moyens de réduire l'hypocalcémie

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Abstract

Calcium is necessary for proper contraction of muscle. Severe hypocalcemia prevents skeletal muscle contraction to the point that the clinical syndrome known as milk fever occurs. While milk fever is a severe form of hypocalcemia, Reinhardt et al., (2011) found more than half of older cows developed subclinical hypocalcemia (blood Ca < 8.0 mg/dl) shortly after calving. Cows with sub-clinical hypocalcemia had elevated concentrations of non-esterified fatty acids compared with normocalcemic cows. Martinez et al (2012) demonstrated that sub-clinical hypocalcemia (blood calcium below 8.6 mg/dl) was a major risk factor for metritis. Cows with sub-clinical hypocalcemia were at a greater risk of developing fever, metritis, and puerperal metritis compared with normocalcemic cows. Prevention of hypocalcemia should be a major goal of all transition cow management and feeding programs.

Résumé

Le calcium est nécessaire à la contraction musculaire. L'hypocalcémie grave empêche la contraction des muscles squelettiques à un point tel que peut apparaître un syndrome clinique appelé la fièvre vitulaire. Bien que la fièvre vitulaire soit une forme grave d'hypocalcémie, Reinhardt *et al.*, (2011) ont découvert que plus de la moitié des vaches plus âgées souffrent d'hypocalcémie subclinique (Ca sanguin < 8,0 mg/dl) peu après le vêlage. Des concentrations d'acides gras non estérifiés plus élevées ont été observées chez les vaches présentant une hypocalcémie subclinique, comparativement aux vaches normocalcémiques. Martinez *et al.* (2012) ont montré que l'hypocalcémie subclinique (Ca sanguin < 8,6 mg/dl) est un facteur de risque majeur de la métrite. Les vaches souffrant d'hypocalcémie subclinique étaient plus à risque de présenter de la fièvre, une métrite et une métrite puerpérale que les vaches normocalcémiques. La prévention de l'hypocalcémie devrait être un des principaux objectifs de tous les programmes de régie et d'alimentation des vaches en transition.

PREVENTING HYPOCALCEMIA

Ca homeostasis is primarily controlled by the parathyroid glands, which respond to hypocalcemia by secreting parathyroid hormone (PTH). The primary target cells are bone osteoblasts and osteocytes, and kidney tubular epithelium cells. PTH action on bone cells is to bring calcium out of bone to support normal blood calcium. PTH action on the kidney is to

reduce urine calcium losses and to stimulate the kidney to produce a hormone derived from vitamin D – 1,25-dihydroxyvitamin D. This hormone activates mechanisms in intestinal cells to enhance absorption of diet calcium. When the system is working normally, there is a small decline in blood calcium at the onset of lactation and then Ca homeostasis kicks into gear and blood calcium returns to normal in a matter of hours.

WHY DOES CALCIUM HOMEOSTASIS FAIL IN SOME COWS?

Mounting evidence suggests the key to milk fever prevention lies in reducing the degree of metabolic alkalosis experienced by the cow just before calving. Cows fed high potassium diets (High DCAD) are in a state of compensated metabolic alkalosis. We fed late gestation cows a High DCAD, alkalinizing diet or a Low DCAD, acidifying diet and treated the cows with synthetic exogenous PTH. The cows fed the alkalinizing diet had a greatly diminished response to the PTH compared to cows fed the acidifying diet. Their kidneys did not produce as much 1,25(OH)₂D and serum Ca did not rise as quickly. It appears the tertiary structure of the PTH receptor is altered during metabolic alkalosis, reducing its affinity for PTH and resulting in a state of pseudohypoparathyroidism (Goff et al., 2014). In highly alkaline cows, despite the fact that bone and kidney cells are exposed to very high concentrations of PTH at the onset of lactation, they respond only poorly to the PTH. Addition of anions to a diet to counteract cations in the diet of a cow reduces the alkalinity of the blood and restores tissue responsiveness to PTH at the onset of lactation. Hypomagnesemia can also interfere with PTH function. It also affects the ability of tissues to respond to PTH and it can also inhibit PTH secretion (Goff, 2014).

DESIRED MINERAL PROFILE OF PRE-PARTUM DIET

The difference between the number of cation and anion particles absorbed from the diet determines the pH of the blood. The cation-anion difference of a diet is commonly described in terms of mEq/kg of just Na, K, Cl, and SO₄ (S) as follows:

$$\text{Dietary Cation-Anion Difference (DCAD)} = (\text{mEq Na}^+ + \text{mEq K}^+) - (\text{mEq Cl}^- + \text{mEq S}^-).$$

This equation is useful, although it must be kept in mind that Ca, Mg, and P absorbed from the diet will also influence blood pH. Evaluation of the relative acidifying activity of dietary Cl vs. SO₄ demonstrates SO₄ is only about 60% as acidifying as Cl (Goff et al., 2004). The DCAD of a diet and its acidifying activity is more accurately described by the following equation: (Na⁺ + K⁺) - (Cl⁻ + 0.6 S⁻). While DCAD equations provide a theoretical basis for dietary manipulation of acid-base status they are not necessary for formulation of mineral content of prepartum dairy cow rations because, with the exception of K and Cl, the rate of inclusion of the other macrominerals can be set at fixed rates.

The NRC (2001) requirement for Na in the diet of a late gestation cow is about 0.12%. A small amount of salt is added to the diet to prevent pica, which often is manifest as a desire to drink urine from the floor. Exceeding the requirement for Na using NaCl is to be avoided in late gestation because it will increase the risk of udder edema, not because it greatly affects acid-base status.

At least two studies have clearly demonstrated that inclusion of Ca in the diet at NRC required levels or several fold above NRC required levels does not influence the degree of hypocalcemia experienced by the cow at calving (Goff and Horst, 1997; Beede et al., 2001). Beede et al. (2001) fed 0.47, 0.98, 1.52, and 1.95 % Ca diets to cows in late gestation being fed a high Cl diet to prevent milk fever. Cows fed 1.5% Ca diets had slightly reduced feed intake when compared to control cows while those fed the 1.95% Ca diet had significantly lower feed intake. Dietary Ca did not influence the degree of hypocalcemia experienced at calving or milk production in the subsequent lactation. It appears from this study that a close-up diet Ca concentration of 1% is optimal. This is similar to the level the cow will receive in the lactating diet and though higher diet Ca may contribute some extra Ca to the blood via the paracellular route of intestinal Ca absorption, higher dietary Ca could negatively impact feed intake. This author has never understood the rationale of feeding high diet Ca (above 1.2% Ca) when the cow's requirement for Ca is easily met by 0.5% Ca diets – and then reducing diet Ca to 0.85-1% when the need for Ca increases in early lactation!!!

Magnesium can only be absorbed from the rumen in cows. Potassium can inhibit the magnesium transporter in the rumen wall. To ensure adequate concentrations of Mg in the blood of the periparturient cow the dietary Mg concentration should be 0.35-0.4% and BIOAVAILABLE. This level of Mg ensures the Mg transporter in the rumen will work even in the face of the inhibitory effects of dietary potassium. This is particularly important in the pre-partum diet and the early lactation diet. Further, hypomagnesemia is the primary cause of mid-lactation milk fever in cows.

Dietary P concentration should be kept low. Diets that supply 0.25% P and are consumed at levels of 13 kg / d or more will supply all the P the cow needs. A diet supplying more than 80 g P/day greatly increases the risk of milk fever. Keeping dietary phosphorus below 40 g / day seems to be safe, though lower levels (35 g /day) improve Ca homeostasis (Peterson, et al., 2005). Keeping diet P low in the prepartum diet does not contribute to the hypophosphatemic downer cow condition, which is sometimes observed as a sequelae of milk fever. We now know that high blood Phosphate concentrations induce production of a hormone, FGF-23, by bone cells, which shuts down the enzyme that produces the active hormonal form of vitamin D. Therefore high phosphate reduces diet Ca absorption.

Dietary S must be kept above 0.22% (to ensure adequate substrate for rumen microbial amino acid synthesis) but below 0.4% (to avoid possible neurological problems associated with S toxicity). Ca sulfate and Mg sulfate are good sources of sulfur that may also supply any needed Mg and Ca.

Now, with the exception of K and Cl, the “variables” in the various proposed DCAD equations have become “fixed”. The key to milk fever prevention (Holstein cows) is to keep K as close to the NRC requirement of the dry cow as possible (about 1.0% diet K). The key to reduction of subclinical hypocalcemia, not just milk fever prevention, is to add Cl⁻ anions to the ration to counteract the effects of even low diet K on blood alkalinity. For formulation purposes the concentration of Cl required in the diet to acidify the cow should first be set at 0.5% less than the concentration of K in the diet. In other words, if diet K can be reduced to 1.3%, the Cl

concentration of the diet should be increased to 0.8%. This will adequately acidify about 20% of herds in this author's experience. Ultimately in many herds the amount of chloride added will have to be brought to within 0.3% of the diet potassium for proper acidification. A conservative approach should be taken when formulating the diet of close-up cows – going immediately to the higher chloride diet will cause over acidification of 20% of herds, which can reduce feed intake creating many other metabolic disease challenges. Move to the higher dose of chloride only if urine acidification (described as a monitoring tool below) is not achieved at the lower chloride level. There is also a limit on how much anion can be added to a diet without affecting feed intake. In this author's experience, when diet potassium exceeds 1.4% it is difficult to add enough chloride to the diet using the traditional chloride salts (Ca, ammonium, and magnesium chloride) to acidify the cow and maintain adequate dry matter intake. With some of the more palatable commercial anion supplements it is possible to acidify the diets and maintain feed intake when diet potassium is as high as 1.8%. If dietary K can only be reduced to 2.0% the diet Cl would need to be roughly 1.5% to acidify the cow. Raising Cl to this level in the diet is likely to cause a decrease in dry matter intake. Chloride and sulfate sources differ in their palatability and since achieving low dietary K can be difficult it is prudent to use a palatable source of Cl or sulfate when formulating the diet. Ammonium chloride (or ammonium sulfate) can be particularly unpalatable when included in rations with a high pH. At the higher pH of high forage (low corn silage) rations where pH of the diet exceeds 5.5, the ammonium cation is converted to ammonia, which is highly irritating when smelled by the cow. Prilling the Cl (and SO₄) salts can reduce the unpleasant taste of the salts and allows improved anion supplementation success. In this author's experience hydrochloric acid has proved the most palatable source of anions as well as the strongest acidifying agent. Hydrochloric acid can be extremely dangerous to handle when it is procured as a liquid concentrate. Several companies now manufacture anion supplements comprised of hydrochloric acid adsorbed onto feed particles, which are safe to handle and palatable.

MONITORING URINE PH

These are simply guidelines for anion supplementation used by this author and are based on inclusion of Ca, Na, S, Mg, and P at the levels outlined above. Urine pH of the cows provides a cheap and fairly accurate assessment of blood pH and can be a good gauge of the appropriate level of anion supplementation. Urine pH on high cation diets is generally above 8.2. Limiting dietary cations will reduce urine pH only a small amount (down to 7.5-7.8). For optimal control of subclinical hypocalcemia the average pH of the urine of Holstein cows should be between 6.2 and 6.8 during the last week of gestation, which essentially requires addition of anions to the ration. If the average urine pH is between 5.0 and 5.5, excessive anions have been added and there is the danger they have induced an uncompensated metabolic acidosis and the cows will suffer a decline in dry matter intake, even if a palatable anion supplement is used.

HYPOMAGNESEMIA

Insufficient dietary Mg supply leads to hypomagnesemia. Hypomagnesemia is a major risk factor for milk fever. Hypomagnesemia affects Ca metabolism by reducing tissue sensitivity to

PTH and by reducing PTH secretion in response to hypocalcemia. Adding Mg to diets before and after calving should eliminate hypomagnesemia as a contributor to hypocalcemia. Unfortunately issues with bioavailability of magnesium sources have arisen.

Magnesium is included in dairy rations for two reasons: to maintain adequate levels of Mg in the blood and as a rumen fluid alkalinizer. Mg sulfate.7 H₂O and Mg chloride.2 H₂O are very soluble, very available sources of Mg. They are acidifying salts of magnesium and are often included in close-up diets as part of a low DCAD diet. Many anion supplements on the market include Mg in the sulfate or chloride form and easily meet the dry cow requirements for magnesium. Once the cow begins lactation the magnesium supplement often switches over to magnesium oxide. MgO takes up little room in the ration, costs less, and is more palatable than some other sources of Mg. It can help alkalinize rumen fluid so it is more appropriately used in lactation diets in conjunction with sodium bicarbonate and other rumen buffers. The feed industry utilizes MgO which is about 54-56% Mg (>58% Mg MgO often indicates the ore was overly heated in the calcining process and the Mg will be poorly available). Unfortunately, there is tremendous variability in MgO quality. For ruminants, MgO should be ground to a fine dust. A quick test can estimate the relative availability of MgO sources. Place 3 g of a MgO source in a container and slowly add 40 ml 5% acetic acid (white vinegar). Cap container and shake well for 15 seconds and let sit. Check the pH after 30 minutes. Vinegar alone is pH 2.6-2.8. The best MgO sources will bring the pH up to 8.2; the worst to just 3.8. pH is a log scale so this represents >10,000 fold difference in the number of hydrogen ions buffered. Remember in lactating rations, MgO is relied upon to combat rumen acidosis- and we are not getting that action from these insoluble MgO sources. In an experiment with four cows with rumen fistulas, the solubility of MgO in vitro (tested in various ways) was found to parallel their solubility in the rumen and their urinary excretion (schonewille, 1998).

ORAL CALCIUM TREATMENTS AT CALVING

Ca administered to the fresh cow may arguably be called a treatment rather than a preventative measure for hypocalcemia. Contrasts between the effects observed with intravenous, subcutaneous, and oral Ca treatments have been described elsewhere (Goff, 1999). Briefly, the concept behind oral supplementation is that the cow's ability to utilize active transport of Ca across intestinal cells is inadequate to help her maintain normal blood Ca concentrations. By dosing the animal with large amounts of very soluble Ca it is possible to force Ca across the intestinal tract by means of passive diffusion between intestinal epithelial cells. Best results are obtained with doses of Ca between 50 and 100 g Ca / dose. Ca chloride has been used but can be very caustic. Ca propionate is less injurious to tissues and has the added benefit of supplying propionate, a gluconeogenic precursor. Ca carbonate is not soluble enough to induce a rapid rise in blood Ca. For best control of hypocalcemia a dose is given at calving and again 12- 24 hrs later. Toxic doses of Ca can be delivered orally – a single dose of 250 g Ca in a soluble form will kill some cows. The benefit of adding oral Ca drenches/gels in addition to a properly formulated low DCAD program is becoming easier to justify as recent studies link even moderate hypocalcemia with decreased health and performance of the cow (Martinez et al., 2012; Chamberlin et al., 2013). It has been estimated that about 4 g Ca entered the blood of cows given 50 g Ca as CaCl₂ in a drench in the first hrs following treatment⁷³.

Author Disclosures

Prior to joining the faculty at Iowa State University, Goff was Director of Research at West Central Farmer's Co-operative. In this capacity he developed anion supplements for prevention of hypocalcemia in cows and Goff continues to consult for this company. Goff also holds a patent using calcium propionate paste to prevent hypocalcemia.

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Conference Colloque



Factors Affecting Energy Intake and Partitioning for Efficient Productive Performance

Facteurs influençant la consommation et la répartition de l'énergie pour favoriser l'efficacité de la performance de production

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Sponsor / Commanditaire:

J.M. Bell Memorial Lecture



Abstract

Understanding factors affecting energy intake and partitioning is key to maximizing animal health and productivity. Feeding behavior is determined by the integration of inhibitory and stimulatory signals by brain feeding centers. These signals are related to various factors including gut distension, osmotic effects, and fuel sensing. Signals transmitted to the brain via hepatic vagal afferents likely affect hunger and satiety depending upon the temporal pattern of fuel oxidation in the liver. The liver is supplied by fuels from the portal drained viscera as well as the general circulation and therefore metabolizes a variety of fuels derived from both the diet and tissues. Effects of nutrients on endocrine response and gene expression affect energy partitioning, which likely affects feeding behavior by altering clearance of fuels from the blood. The type and temporal supply of fuels to the liver varies with diet composition and physiological state of animals. Dominant mechanisms controlling feed intake change with physiological state, which is highly variable, especially through the lactation cycle. Variation among species in type and temporal supply of fuels to the liver as well as differences in hepatic uptake and metabolism of fuels allows insight into this mechanism. The liver is an ideal candidate to sense energy status because it is supplied by fuels provided by the diet and mobilized from body stores and is likely a primary energy sensor, conveying a signal of overall energy status to brain feeding centers. Mechanisms controlling energy intake and partitioning are entwined and inseparable and are affected by both diet and physiological state of animals.

Résumé

Comprendre les facteurs qui interviennent dans la consommation et la répartition de l'énergie est essentiel pour maximiser la santé et la productivité des animaux. Le comportement alimentaire est déterminé par l'intégration des signaux d'inhibition et de stimulation envoyés par les centres de la faim du cerveau. Ces signaux sont associés à divers facteurs, dont la distension du tube digestif, les effets osmotiques et les mécanismes de détection de l'énergie. Les signaux transmis au cerveau par les voies afférentes vagues hépatiques interviennent probablement sur la faim et la satiété, dépendamment du régime temporel de l'oxydation des combustibles dans le foie. Le foie est alimenté par des combustibles provenant des viscères drainés par la veine porte ainsi que de la circulation générale et, conséquemment, il métabolise divers combustibles issus des aliments et des tissus. Les effets des nutriments sur la réponse endocrinienne et l'expression génique interviennent sur la répartition de l'énergie, ce qui influence probablement le comportement alimentaire et modifie le rythme d'élimination des combustibles dans le sang. Le type de combustibles fournis au foie et son approvisionnement dans le temps varient avec la composition de la ration et l'état physiologique des animaux. La dominance des mécanismes de contrôle de la consommation alimentaire change avec l'état physiologique, qui est lui-même très variable, surtout pendant le cycle de lactation. La variabilité entre les espèces quant au type de combustibles fournis au foie et à son approvisionnement dans le temps ainsi que les différences quand à l'absorption et au métabolisme des combustibles dans le foie permettent de mieux comprendre ce mécanisme. Le foie est un candidat idéal pour capter l'état énergétique, parce qu'il est alimenté par des combustibles provenant de la ration alimentaire et mobilisés des réserves corporelles et qu'il est probablement un des principaux détecteurs d'énergie transmettant un signal de l'état énergétique général aux centres de la faim du cerveau. Les mécanismes de contrôle de la consommation et de la répartition de l'énergie sont dépendants les uns des autres et indissociables, et ils sont influencés à la fois par la ration alimentaire et l'état physiologique des animaux.

Introduction

Understanding factors that affect feed intake and partitioning of nutrients is essential for optimal diet formulation. Feed intake is positively related to energy requirements which can be predicted with reasonable accuracy but is also greatly affected by diet. In addition, diet effects vary with the physiological state of animals. For instance, DMI is likely controlled by metabolic mechanisms in the postpartum period for lactating cows but as lactation proceeds, gut distention by digesta begins to dominate control of feed intake. In addition, physiological state affects the partitioning of fuels among tissues and therefore the availability of fuels for oxidation in the liver and feeding behavior. The objective of this paper is to discuss the control of feed intake by farm animals. Much of the research for the metabolic control of feed intake was with laboratory species with more recent research with ruminants. Variation among species in type and temporal supply of fuels to the liver as well as differences in hepatic uptake and metabolism of fuels provides strong support that feeding is controlled by the hepatic oxidation of fuels. The interdependence of mechanisms controlling energy intake and partitioning is discussed with research with lactating cows as they change physiological state through lactation. Lastly, research with ruminants is used to discuss factors affecting the filling effects diets and its effect on feed intake.

Metabolic Control

Mayer (1953) proposed the glucostatic theory of the control of food intake based upon research with non-ruminant species. However, glucose has little effect on feed intake of ruminants; glucose infused intravenously in cows, intraperitoneally in heifers, intracerebroventricularly in calves, abomasally in lactating cows, and intraportally in sheep had no effect on feed intake (reviewed by Allen, 2000). Booth (1972) proposed the “energostatic” control of food intake, suggesting that animals eat to balance energy consumed with energy required. However, the energostatic theory fails to explain the suppression of appetite and extended negative energy balance for some mammals (e.g. cows, sows) in the peripartum period that begins a week or more prepartum. The idea that feed intake is limited by energy supplied in excess of energy demand has some merit but the mechanism is complex and only loosely controlled when based on energy *per se*. Infusion of various fuels derived from the diet (e.g. glucose, acetic acid, propionic acid) revealed differences in the effects of fuels on both dry matter and energy intake (Allen, 2000). Hypophagic effects of propionate infusions have been documented extensively for ruminants (reviewed by Allen, 2000). Propionate is more hypophagic than acetate or butyrate when infused into the portal vein of sheep (Anil and Forbes, 1980), and infusion of propionate into the mesenteric vein of steers reduced feed intake, whereas acetate infused at similar rates did not (Elliot et al., 1985). Although propionate might be expected to decrease feed intake compared with acetate because it has higher energy content, propionate linearly decreased metabolizable energy intake compared with acetate in lactating cows when infused intraruminally as iso-osmotic mixtures (Oba and Allen, 2003c). These studies suggest that it is unlikely that animals consume feed to meet their energy requirements *per se* but rather have fuel-specific mechanisms regulating satiety and hunger.

Signal from the liver Russek (1963) introduced the idea that the liver is involved in the control of food intake. The liver is in a unique position to monitor changes in fuel metabolism to control eating behavior because of its central role in energy metabolism of animals (Freidman and Stricker, 1976). It is likely involved in regulation of intake because hepatic vagotomy eliminated hypophagic effects of propionate infusion in sheep (Anil and Forbes, 1988) and fatty acid oxidation in rats (Sharrer, 1999). Research with laboratory species suggests that meals can be terminated by a signal carried from the liver to the brain via afferents in the vagus nerve that are affected by hepatic oxidation of fuels (Langhans and Sharrer, 1992) and generation of ATP (Friedman, 1995). The signal from the liver might be both stimulatory and inhibitory, depending upon the firing rate of vagal afferents; firing rate is increased as energy status decreases, stimulating feeding, and is decreased when energy status increases, inhibiting feeding (Friedman 1997). Evidence that feeding is related to hepatic energy status was discussed by Friedman (1997) using rats as a model: 1) hepatic ATP concentration is decreased by fasting and increased by re-feeding, 2) prevention of ATP production by trapping inorganic phosphate stimulates feeding, while phosphate loading reverses the effects on ATP and feeding, 3) trapping the adenine moiety of ATP by ethionine also stimulates feeding, indicating that the mechanism affecting feeding is related to ATP *per se* and not inorganic phosphate, 4) the decline in liver ATP by trapping inorganic phosphate coincided temporally with the feeding response, and 5) the probability of meal initiation was negatively related to liver ATP concentration. While energy

utilization by the liver is likely consistent over the short term of days, oxidation of fuels and production of ATP over minutes can vary greatly, affecting feeding behavior and feed intake. Therefore, normal fluctuations in liver ATP concentration within days might stimulate both hunger and satiety depending upon the balance between energy production and utilization. We call this the Hepatic Oxidation Theory (HOT) of the control of feed intake and have applied it to ruminants.

Hepatic oxidation Acetyl CoA is the metabolic crossroad that all fuels must be converted to for oxidation. Some fuels are anaplerotic and can stimulate oxidation of acetyl CoA in the TCA cycle. Anaplerotic fuels in non-ruminant species include glucose, glucogenic amino acids, glycerol, and lactate. Hepatic removal of glucose is negligible in mature ruminants (Stangassinger and Giesecke, 1986) likely because hexokinase activity is low in ruminants compared with nonruminants (Ballard, 1965), consistent with their differences in the hypophagic effects of glucose. Also, propionate produced by ruminal fermentation is a primary J. Dairy Sci. 82:589-596.anaplerotic fuel for ruminants and is likely the primary fuel stimulating satiety because its flux to the liver increases greatly during meals (Benson et al., 2002). While propionate is extensively metabolized by the ruminant liver, there is little net metabolism of acetate (Reynolds, 1995). Ruminant liver has high activity of propionyl CoA synthetase but not acetyl CoA synthetase (Demigne et al., 1986; Ricks and Cook, 1981) necessary for activation and subsequent metabolism, thus explaining differences in hypophagic effects of infusions of propionate and acetate in ruminants.

Although butyrate can contribute to hepatic oxidation, net metabolism is generally less than propionate because ruminal production is lower and butyrate is preferentially oxidized by ruminal epithelia (Weigand et al., 1975). Glycerol, like propionate, is a glucose precursor but enters the gluconeogenic pathway at glyceraldehyde-3-phosphate and is less likely to enter the TCA cycle, stimulating oxidation. Propionic acid decreased feed intake 17% relative to glycerol by decreasing meal size when isoenergetic solutions were infused abomasally in cows in the postpartum period, consistent with the HOT (Gualdron-Duarte and Allen 2017).

Cereal grains that are highly digestible in the rumen can depress feed intake of lactating cows; feed intake was depressed nearly 3 kg DM/d (~13%) when more fermentable grains were substituted in diets of lactating cows in several studies reported in the literature (Allen, 2000). Depression in feed intake by highly fermentable starch sources likely occurs because greater ruminal propionate flux to the liver stimulates hepatic oxidation and generation of ATP reducing meal size. In contrast, when site of starch digestion is shifted post-ruuminally, a positive response in feed intake is likely. This is because the fuels absorbed do not stimulate hepatic oxidation to the same degree as propionate, and because of increased latency for fuels reaching the liver; transit time from the rumen to the intestines significantly delays fuel absorption. Starch escaping the rumen is digested to glucose, which is absorbed and partially metabolized to lactate. Lactate produced from metabolism of glucose in the intestines is expected to have considerably less effect on satiety than propionate because liver uptake of lactate is much lower than propionate (Reynolds et al., 2003). Abomasal infusion of propionic acid decreased total metabolizable energy intake (diet plus infusion) compared with control (no infusion) from 40.1 to 34.8 Mcal/d but iso-energetic abomasal infusions of glucose and lactic acid did not decrease metabolizable energy intake compared with control (Gualdron and Allen, 2018). Whereas the ability of fuels to

stimulate hepatic oxidation is consistent with their hypophagic effects, feeding behavior response to diet is also affected by physiological state.

Interaction with physiological state Differences in fermentability of diets and metabolic state of animals likely contributed to inconsistent effects of propionate infusion on feed intake reported in the literature (Allen, 2000). Variation in the hypophagic effects of propionate might be related to the balance between flux of propionate to the liver and rate of utilization of propionate for gluconeogenesis, affected by glucose demand (Allen, 2000). The maximum rate of gluconeogenesis at any point in time is regulated by hormones such as insulin and glucagon, which are affected by plasma glucose concentration (McGrane, 2000). Increased glucose demand is expected to increase gluconeogenic capacity by lowering plasma glucose and decreasing the ratio of insulin to glucagon (McGrane, 2000). According to the HOT, when glucose demand is high, gluconeogenesis increases, increasing cataplerosis and decreasing TCA cycle activity and oxidation of acetyl CoA, resulting in greater meal size. Conversely, when glucose demand is low, TCA activity is increased generating ATP, resulting in satiety and smaller meal size. In support of this, the marginal depression in feed intake from propionate infusion was positively related to plasma glucose concentration of cows (Oba and Allen, 2003b). Because these enzymes are hormonally regulated, insulin and glucagon likely provide the link between glucose demand and hepatic nutrient flux.

Insulin concentration over the long term (days, weeks) indicates energy adequacy, affecting tissue uptake or mobilization of fuels and regulating their oxidation in the TCA cycle through its effects on gluconeogenesis. However, release of insulin at meals might have opposite effects on feed intake. Pulsatile insulin secretion in response to feeding might clear fuels from the blood more quickly, potentially decreasing the interval between meals (Oba and Allen, 2000). While the extent of feed intake depression by a more fermentable diet was related positively to plasma insulin concentration, consistent with effects of insulin on gluconeogenesis, it was related negatively to insulin response to an intravenous glucose challenge ($r^2 = 0.40$, $P < 0.01$ quadratic); cows with greater insulin secretion from the glucose infusion were better able to maintain feed intake on the more fermentable diet (Bradford and Allen 2007). Production of acetyl CoA by β -oxidation is dependent upon supply of NEFA to the liver, which is reduced post-prandially by effects of absorbed fuels on insulin secretion (Allen, 2014). The reduction in NEFA supply to the liver during meals, affected by insulin secretion and sensitivity of adipose tissue, might affect feeding behavior response to absorbed fuels by reducing acetyl CoA available for oxidation. Hepatic acetyl CoA concentration decreased over the first 4 hours following feeding corresponding to a decrease in plasma NEFA concentration, and cows with greatest DMI over the first 4 h after feeding had greatest decrease in both plasma NEFA and hepatic acetyl CoA concentration (Piantoni *et al.* 2015).

Temporal variation in the concentration of amino acids in blood likely contributes to control of feeding behavior by their metabolism in the liver. Insulin stimulates uptake of amino acids by muscle tissue and inhibits protein degradation (Lobley 1992) thus affecting supply of amino acids to the liver. Mobilization of amino acids from muscle occurs during negative energy balance when plasma insulin concentration is low (Heitmann and Bergman 1980). Amino acids can be oxidized in the TCA cycle through acetyl CoA and most (anapleurotic) amino acids can

stimulate oxidation of acetyl CoA. Amino acid imbalances increase their potential for deamination and oxidation, contributing to satiety.

Hyperlipidemia in the periparturient period is caused by a reduction in plasma insulin concentration combined with a reduction in insulin sensitivity of adipose tissues (Allen et al., 2009). Uptake of NEFA by the liver increases greatly (Reynolds et al., 2003), resulting in increased FA oxidation and the buildup of acetyl CoA results in hepatic export of ketones. According to the HOT, feed intake might be suppressed by hepatic fatty acid oxidation and generation of ATP. Concentration of acetyl CoA in hepatocytes varies by physiological state (Stocks and Allen 2012) as well as diurnally (Piantoni *et al.* 2015). The primary source of acetyl CoA is from mitochondrial β -oxidation of NEFA but all fuels (lactate, glycerol, AA) that are completely oxidized in the mitochondria must enter the TCA cycle via metabolism to acetyl CoA. Hypophagic effects of anapleurotic metabolites appear to be dependent upon the availability of acetyl CoA to be oxidized. We reported that hypophagic effects of intraruminal infusions of propionic acid were linearly related to hepatic concentration of acetyl CoA for cows in the postpartum period (Stocks and Allen 2012) and that the hypophagic effects were not attenuated over a 3-d infusion (Stocks and Allen 2013). This is consistent with previous results from our laboratory in which propionic acid was more hypophagic for cows in the postpartum period compared with cows in mid-lactation (Oba and Allen 2003a); cows in the postpartum period were in a lipolytic state with elevated plasma BHBA, indicating elevated hepatic acetyl CoA concentrations, while cows in mid-lactation were in positive energy balance with low plasma concentration of BHBA.

Physical Limitations

Physiological state and nutrient requirements vary greatly through lactation. Diets for ruminants include forages for proper rumen function, and forage fiber digests and passes from the rumen more slowly than other diet components. Therefore, feed intake can be limited by distention of undigested residues in the gastrointestinal tract. In the immediate postpartum period, dairy cows are in negative energy balance but neither the filling effect, nor energy content of diets can be altered to eliminate this. Distention likely begins to control of feed intake when milk yield and energy requirements increase in the weeks following parturition and likely limits feed intake through peak lactation. As milk yield declines through lactation, distention becomes less limiting and mechanisms related to specific fuels dominate control of feed intake.

The reticulorumen is generally regarded as the site within the gastrointestinal tract at which distention limits feed intake (Campling, 1970; Baile and Forbes, 1974). Tension receptors that respond to distention are located primarily in the reticulum and cranial sac (Leek, 1986). The mechanism by which distention limits feed intake is likely via transmission of signals from these tension receptors to brain feeding centers rather than a limitation by volume *per se*. This is supported by dose-dependent decreases in feed intake from additions of inert fill (water or air-filled bladders, plastic cubes, etc.) into the reticulorumen in several experiments (Allen, 1996). In addition, distension in the reticulorumen is determined by both volume and weight of contents; dry matter intake was reduced 112 g for each kilogram of weight and 157 g for each liter of volume that was added to the reticulorumen as inert fill (Schettini et al., 1999).

Furthermore, we reported a reserve volume of 16 L in the reticulorumen of lactating cows consuming a fill-limiting diet with addition of 22 L of inert fill, indicating that additional capacity for volume may exist even when distention in the reticulorumen limits feed intake (Dado and Allen, 1995).

The abomasum might also be involved in the physical limitation of feed intake. Abomasal infusion of methylcellulose decreased feed intake by sheep (Grovmum and Phillips, 1978) and distention of the abomasum of sheep with water filled bladders resulted in a dose dependent reduction in feed intake (Grovmum, 1979). In addition, increasing abomasal emptying rate by suppressing the pyloric gate by pylorotomy and pyloroplasty, increased feed intake by sheep (Malbert and Ruckebush, 1989). Abomasal distention might generate a satiety signal because mechanoreceptors in abomasal epithelium have been described that discharge with distention (Harding and Leek, 1972). Alternatively, restricted flow from the abomasum might limit feed intake by decreasing flow from the rumen and increasing ruminal distention.

Although many studies have shown decreases in feed intake with added inert fill, results have not been consistent across experiments (Allen, 1996). The extent of reduction in feed intake depends upon the extent to which feed intake is limited by distention before the addition of inert fill; animals with greater energy requirements consuming low energy, high fill diets are affected the greatest extent (Allen, 1996). Regardless of the site at which the signal is generated, it is clear that feed intake can be limited by distention within the gastrointestinal tract the extent of limitation is dependent upon the filling effect of the diet.

The filling effect of forages was reported to be related to fiber mass (Balch and Campling, 1962) and feed intake of forages was more highly related to their neutral detergent fiber (NDF) content than to other chemical measures (Van Soest, 1965). Although the NDF content has been used as the only diet characteristic to predict feed intake by dairy cattle consuming mixed diets of forages and concentrates (Mertens, 1987, 1994), there is substantial evidence that NDF alone is inadequate.

Forage NDF has a much longer ruminal retention time than other major dietary components including NDF from other sources. Retention time in the rumen is longer because of longer initial particle size, and greater buoyancy in the rumen over time (Allen, 2000). Increasing diet forage NDF concentration can dramatically reduce feed intake of high producing cows. Several studies in the literature reported a decrease in DMI of up to 4 kg/d when diet NDF concentration was increased from 25 to 35% by substituting forages for concentrates (Allen, 2000). Although most studies reported a significant decrease in DMI as forage NDF increased, the DMI response was variable, depending upon the degree to which intake was limited by undigested feed residues in the rumen.

Increasing diet NDF content by substituting non-forage fiber sources (NFFS) for concentrate feeds has shown little effect on DMI in studies reported in the literature (Allen, 2000). Non-forage fiber sources include byproduct feeds with higher concentrations of NDF such as soyhulls, beet pulp, cottonseeds, corn gluten feed, almond hulls and distiller's grains. Fiber in NFFS is less filling than forage NDF because it is less filling both initially (smaller particle size) and over time in the rumen because it digests and passes from the rumen more quickly.

Therefore, the forage NDF content of mixed diets containing both forages and concentrates is a more important measure than total NDF content.

The chemical composition of forage NDF varies greatly and affects its susceptibility to enzymatic degradation. Digestibility of NDF varies among forage type (e.g. grasses vs. legumes) and decreases as forages mature and become more lignified. We reported that greater NDF digestibility of forages measured in vitro or in situ was related positively to dry matter intake and milk yield of lactating cows; a one unit increase in NDF digestibility corresponded to a 0.17 kg/d increase in DMI and 0.25 kg/d increase in fat-corrected milk (Oba and Allen, 1999b). Within a forage type, NDF that is more fermentable clears from the rumen faster and is less filling, allowing greater feed intake when limited by distention. However, this applies only within forage type; NDF from perennial grasses is generally much more digestible than NDF from legumes but is also more filling and more likely to limit feed intake as discussed below.

The filling effect of forage NDF is also affected by initial particle size; decreasing particle size of forages by grinding and pelleting generally increases feed intake with a greater intake response by pelleting low-quality forages compared with high-quality forages (Minson, 1963). Long forage particles are required to form the rumen mat that functions to selectively retain small fibrous particles in the reticulorumen, increasing their digestibility (Allen, 1996). However, experiments that have evaluated effects of forage particle size have generally shown only small effects on DMI of lactating cows (Allen, 2000), probably because initial particle size was sufficient to form a rumen mat.

The filling effect of forage NDF over time is affected by the fragility of forage particles. Forage fragility varies greatly and affects the rate of reduction in particle size from chewing during eating and ruminating (Poppi et al., 1981). Faster particle size reduction will increase the mass of particles below the threshold size to pass from the reticulorumen as well as decrease the ability of the rumen to selectively retain those particles by decreasing the size of the rumen mat. Although NDF of perennial grasses is less lignified and more digestible than NDF of legumes, feed intake is generally lower for perennial grasses than legumes (Oba and Allen, 1999b). Grasses are more filling because retention time in the rumen is greater (Voelker Linton and Allen, 2008; Kammes and Allen, 2012a&b). Kammes and Allen (2012a) reported that ruminal retention of orchardgrass NDF was longer than alfalfa NDF because it was more resistant to particle size breakdown despite greater rumination time per kg NDF.

In the decades since the first observations that feed intake can be limited by distention from undigested feed residues within the gastrointestinal tract in ruminants, we have learned that the overall filling effect of diets is determined primarily by forage NDF content, forage particle size, fragility of forage NDF determined by forage type (legumes, perennial grasses, annual grasses), and NDF digestibility within a forage family (Allen, 2000).

Integration of Signals

The signal from the liver and other peripheral signals are relayed to brain feeding centers by sensory nerves and integrated to affect feeding behavior. The relative contribution of different

signals likely varies temporally, within and across day]\Synergistic effects of signals have been demonstrated for ruminants with additive effects of intraruminal infusion of short chain fatty acids on feed intake of lactating cows when rumens were distended by balloons (Mbanya et al. 1993) and forage NDF concentration of the diet (Choi and Allen 1999). Therefore, mechanisms are not mutually exclusive and different mechanisms can both contribute to, or dominate, control of feeding within days.

Conclusions

The effects of diet on feed intake is affected by energy requirements of animals and the interaction between the diet and the physiological state of animals. Feed intake can be limited by distention of undigested feed residues within the gastrointestinal tract especially when energy requirements are high or when diets are filling. The filling effect of diets is related to the content of forage NDF and the initial size, fragility, and digestion characteristics of forage particles. The liver is likely a primary energy sensor, conveying a signal of overall energy status to brain feeding centers. It is an ideal candidate to sense energy status because it is supplied by fuels provided by the diet and mobilized from body stores. Therefore, mechanisms controlling energy intake and partitioning are entwined and inseparable and are affected by both diet and physiological state of cows.

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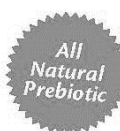
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Role of Livestock Sector in Global Sustainability

Le rôle du secteur de l'élevage dans le développement durable global

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Abstract

The demand for livestock products is expected to grow substantially and the challenge of the 21st century is to ensure this happens sustainably and equitably. Livestock provides nutrient dense product to human diet. The greatest gap in meeting the requirement in developing countries (e.g. Cameroon) and the US was dairy products. The challenge is how does one evaluate livestock's contribution to human diet? If only calorie or protein are used, animal source food (ASF) seem less sustainable. However, using 6 or 15 essential nutrients, ASF are more sustainable compared to grains and other sources. Another perspective is that livestock consume up to 86% of feed that human inedible. Therefore, based on human edible food intake, dairy and beef add to our food supply. Sustainability is usually viewed as a wicked problem with triple bottom line of environment, economic and social considerations. Environmentally, the main issue is greenhouse gas (GHG) emissions. However, there are some challenges in quantification which require standardization globally. Nutrient flows are also a concern and there may be a tradeoff between GHG emissions and nutrient excretion. Therefore, a method that takes into consideration several impact categories such as Life cycle assessment (LCA) is essential. Production of ASF will require estimates of emissions from feed preparation (including specialty feed ingredients), animal husbandry and manure management. Due to regional variability, local sources of emissions need to be properly estimated within LCA approach. Considerations must also be given for socio-economic sustainability. The social concerns are different in developing and developed countries with different solutions to those challenges. Economic and environmental sustainability may or may not be compatible depending on the production system assessed. In summary, redesigning sustainable livestock farming systems is a challenge balancing multiple and changing objectives and assessment must be conducted in a standardized manner.

Résumé

La demande pour les produits d'origine animale devrait croître substantiellement, et le défi au XXI^e siècle sera d'y répondre de façon durable et équitable. Le bétail fournit des aliments à densité élevée en nutriments aux humains. C'est au chapitre des produits laitiers que l'écart pour combler les besoins dans les pays en développement (p. ex., Cameroun) et aux É.-U. est le plus grand. La difficulté est de savoir comment chacun évalue la contribution du bétail dans l'alimentation humaine. Quand seules les calories et les protéines sont considérées, les aliments d'origine animale (AOA) s'accordent moins bien avec le développement durable. Toutefois, si on tient compte de 6 ou 15 nutriments essentiels, les AOA sont plus durables que les grains et d'autres sources. Une approche différente est de considérer que le bétail consomme jusqu'à 86 %

d'aliments non comestibles pour l'homme. Par conséquent, compte tenu de la consommation d'aliments comestibles pour l'homme, les bovins laitiers et de boucherie augmentent notre approvisionnement alimentaire. Le développement durable est généralement considéré comme un problème pernicieux qui trouve sa solution dans des considérations environnementales, économiques et sociales. Au plan de l'environnement, le principal problème sont les émissions de gaz à effet de serre (GES). Cependant, leur quantification pose certains défis qui demanderont une standardisation à l'échelle planétaire. Les flux de nutriments soulèvent également des préoccupations, et il pourrait y avoir compromis entre les émissions de GES et l'excrétion de nutriments. Par conséquent, une méthode qui prend en considération plusieurs catégories de répercussions, telle que l'analyse du cycle de vie (ACV), est indispensable. La production d'AOA devra se baser sur des évaluations des émissions issues de la préparation des aliments (incluant des ingrédients alimentaires de spécialité), de l'élevage et de la gestion des déjections. En raison de la variabilité régionale, les sources locales d'émissions doivent être évaluées correctement dans le contexte de l'approche de l'ACV. Le volet socioéconomique du développement durable doit aussi être considéré. Les problèmes sociaux dans les pays en développement ne sont pas les mêmes que dans les pays développés et demandent des solutions différentes. Le développement économique durable et le développement économique environnemental peuvent ou non être compatibles, selon le système de production évalué. En résumé, recréer des systèmes durables de production animale est un défi qui demande de jongler avec des objectifs multiples et changeants, et l'évaluation doit être effectuée à partir de valeurs standardisées.

Introduction

The challenge of the 21st century is that can we stay on trend in food and agriculture? Or are we facing tightening global food supply-demand balance? Is there a reversal of the favorable trend in food supply per capita? Or increasing risk of food price spikes? We know with relative certainty that (1) global population will be exceeding 9 billion around 2050, (2) rising average incomes, (3) resource competition and scarcity and (4) environmental change (Godfray and Garnett, 2014). However, many big uncertainties remain such as climate change and compounded uncertainties on yields, water supply, invasive pests and diseases, and policy responses to climate change.

Diets are expanding and shifting. Sugar, fat, and animal product consumption are increasing in almost all regions of the world—yet people in low- and middle-income countries still consume far less meat and dairy than those in high-income countries (Kastner et al., 2012). By 2050, Latin America, Near East/North Africa and East Asia will have a per capita food consumption similar to that of high-income countries in 1990 (Alexandratos and Bruinsma 2012). The production of animal protein must be more than tripled if the projected global population of 9 billion people in 2050 were to consume meat and dairy at current North America and Europe levels (PBL 2009). This article addresses the questions can the increase be achieved in a sustainable manner? What are the implications for the environment? How can environmental sustainability be assessed? How can we compare animal source food (ASF) with plant based food sources for their impact on the environment?

Sustainable Development Goals and Evaluation of Livestock's Contribution to Human Diet

Livestock are central to achieving many of the United Nations Sustainable Development Goals (SDGs) and directly relevant to most of them. The growing demand for livestock products in developing countries, driven by population growth, higher incomes and urbanization, represents a huge opportunity for hundreds of millions of poor smallholder livestock farmers, processors and marketers, many of whom are women, to meet that market demand and rise out of poverty. Livestock products (meat, milk, eggs) provide essential nutrients that contribute to food and nutritional security (ILRI, 2018). One way to frame sustainability is as a 'wicked problem' that cannot be solved, only managed. It is generally accepted that it involves 'triple bottom line' including social, environmental and economic considerations. This article will be more focused on environmental considerations and evaluation of ASF to human diet.

Comparison of daily per capita energy of Cameroon's food supply with the 'ideal' diet to meet requirement showed that the largest gap is lack of dairy, meat, poultry, fish and eggs (Kuyper et al. 2017). The same study showed oversupply of grains, fats and oils and nuts in the Cameroonian diet. Therefore, in order to meet their requirements the study group need to consume much more ASF. Similarly, the dietary guidelines for Americans shows that over 80% under consume dairy while 70-90% overconsume sugars, saturated fat and sodium (USDA 2015). How should livestock's contribution to human diet be assessed? In the literature it is mostly based on calorie and/or protein content. However, this is too simplistic because it does not account for the variety of easily available nutrients in animal products. Drewnowski et al. (2015) suggested that considerations of the environmental impact of foods need to be linked to concerns about nutrient density and health. Taking up to either 6 or 15 nutrients per 100 kcal in foods showed that ASF have considerably lower greenhouse gas emissions compared to plant based food.

Food vs Feed Competition

Livestock consume about 6 billion tonnes DM as feed per year, of which 86% is made of materials that are either not human edible or not eaten by humans. In addition, livestock play a key role in the bio-economy by converting forages, crop residues and agricultural by-products into high-value products and services. The production of global feed requires 2.5 billion ha of land, which is about half of the global agricultural area. Most of this area, 2 billion ha, is grassland, of which about 1.3 billion ha cannot be converted to cropland (rangeland). This means that 57% of the land used for feed production is not suitable for food production (Mottet et al. 2017). For dairy cattle, the return on human edible protein inputs (calculated as the output of human edible protein in products compared with human edible protein input with feed) is larger than 1 (range: 1.4 to infinite; infinite if diet contains no human edible protein), indicating that dairy cattle add to the total human food supply. For beef cattle, protein efficiencies on a human-edible basis are also often larger than 1 but are more variable (range: 0.33 to infinite) than for dairy cattle. The ability of ruminants to turn fibrous feed resources into edible animal food of high biological value is likely to become of greater significance in terms of global human food

production as the population of the planet and demand for human-edible plant resources increases rapidly (Dijkstra et al., 2013).

Holistic Assessment

Production of ASF or any other food impacts the environment in several ways. Therefore a proper impact analysis should involve the main categories including climate change. The carbon cost is by no means the only measure of sustainable agriculture. Because of concerns regarding the impact of agricultural production on global climate change, it is important to develop additional metrics to assess crop biodiversity and the use of water resources and land-use change. For example, Mekonnen and Hoekstra (2012) calculated that production of eggs and beef to require lower water consumption than some fruits and nuts. Life cycle assessment (LCA) technique is becoming the gold standard in environmental impact measurement. However, methodological choices and assumptions may be subjective and affect the results. Delineation of system boundary, functional units, and allocation techniques must be carefully considered, particularly when comparing studies. For example, Kebreab et al. (2016) quantified the role of feed additives, particularly amino acids and phytase in environmental sustainability of swine and poultry production. Their analysis showed that in both swine and poultry using crystalline amino acids reduced greenhouse gas emissions, eutrophication and acidification potentials. However, if land use change were to be considered, European and South American production systems showed drastic reduction in greenhouse gas emissions by using feed additives because it relieves the production of soybeans.

Summary and Conclusions

Demand will drive increased production of ASF so sustainable production is a must. There is no one shoe fits all solution and each production system must consider multiple options. Redesigning sustainable livestock farming systems is a challenge balancing multiple and changing objectives. The aim is for a *more sustainable* system as sustainability is not a fixed state. To evaluate sustainability (at least environmental), we need to have a standard system of accounting such as those developed by the UN FAO Livestock Environmental Assessment and Performance partnership and consider macro and micronutrients in ASF in addition to calories and protein. Policy dialogue through multi-stakeholders initiative e.g. the global agenda for sustainable livestock enables discussion with all stakeholders involved in livestock supply chains to build common knowledge and solutions.

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Modeling and Integrating Metabolizable Energy and Protein Supply and Requirements in Dry and Lactating Dairy Cattle to Optimize Nitrogen Utilization

Modélisation et intégration de l'apport et des besoins en énergie métabolisable et protéines chez les bovins laitiers au tarissement et en lactation pour optimiser l'utilisation de l'azote

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Abstract

The dairy cow has two requirements, one for rumen microbial function and growth that can be supplied by ammonia, small peptides and amino acids (AA) whereas the other requirement is for post-ruminal AA. Ruminant requirements are met from feed nitrogen (N), recycled urea and endogenous protein. Current data on urea recycling indicates the cow converts 50 to 70% of intake N into ammonia, which is converted to urea and of that 35% of intake N via urea is transferred into the rumen. The remaining urea N is excreted in the urine and milk. In addition to urea, the cow recycles a significant amount of endogenous protein that is utilized by the microbes or flows to the small intestine as high quality AA. The endogenous protein can constitute 20% of the total AA supply to the cow, has a high intestinal digestibility (ID) and until recently this has been ignored and considered only as a maintenance requirement. Further, to ensure adequate N availability at the small intestine, the digestibility in the intestine needs to be considered. Recent data suggest feeds like blood meal can vary from 40% to 90% intestinal digestibility and not knowing the ID would create a situation where productivity falls, thus reducing the efficiency of use of absorbed N. Finally, energy drives the efficiency of use of absorbed AA and the AA are best described as a function of the metabolizable energy (ME) intake of the cow as ME drives protein synthesis, thus creating the demand for AA.

Résumé

La vache laitière a deux besoins, un pour assurer l'activité et la croissance microbiennes dans le rumen et qui peut être comblé par l'ammoniac, les petits peptides et les acides aminés (AA) et un

pour les AA en aval du rumen. Les besoins du rumen sont comblés à partir de l'azote (N) des aliments, de l'urée recyclée et des protéines endogènes. Les données actuelles sur le recyclage de l'urée indiquent que la vache convertit 50 % à 70 % du N consommé en ammoniac, lequel est converti en urée, et que, par la suite, 35 % du N de l'urée consommé passe dans le rumen. Le reste de l'azote uréique est excrété dans l'urine et le lait. En plus de l'urée, la vache recycle une quantité importante de protéines endogènes qui sont utilisées par les microorganismes ou qui passent dans le petit intestin où elles constituent une source d'AA de haute qualité. Les protéines endogènes peuvent fournir jusqu'à 20 % des AA totaux de la vache et présentent une digestibilité intestinale élevée; jusqu'à tout récemment, ce phénomène avait été ignoré et considéré seulement dans le contexte des besoins d'entretien. En outre, pour garantir une disponibilité en N adéquate dans le petit intestin, la digestibilité dans l'intestin doit être prise en compte. Des données récentes suggèrent que la digestibilité intestinale de certains aliments comme la farine de sang peut varier de 40 % à 90 % et que d'ignorer la digestibilité intestinale pourrait créer une situation de perte de productivité, diminuant ainsi l'efficacité de l'utilisation du N absorbé. Enfin, l'énergie contrôle l'efficacité de l'utilisation des AA absorbés, et il est préférable de décrire les AA comme une fonction de la consommation d'énergie métabolisable (ÉM) de la vache, puisque l'ÉM contrôle la synthèse protéique, créant ainsi une demande en AA.

Introduction

Improving the prediction of supply and use of metabolizable energy (ME) and protein (MP) is dependent on several factors that can be measured routinely or predicted with reasonable precision. The prediction of ME is dependent on factors such as total feed intake, the chemical composition of the feed consumed, and ruminal and post-ruminal digestibility. The prediction of MP is dependent on the same factors, although MP is more complex as it is highly dependent on the quantity, profile and digestibility of amino acids that escapes the rumen, whereas substrates for ME can be absorbed anywhere along the GIT, recognizing how those substrates are partitioned are different as they are absorbed farther down the GIT. Feed protein is one of the most expensive macronutrients in dairy cattle rations, and overfeeding degradable protein relative to supply results in excessive N losses to the environment (Huhtanen and Hristov, 2009). Efficient use of feed N can be achieved by first meeting the requirements of the rumen microbial population, followed by balancing diets to meet the amino acid requirements of the cow. To decrease competition for quality protein that could otherwise be fed to humans, dairy cattle can be fed byproducts of human food production, thereby converting waste product streams into highly valuable milk protein.

To frame the discussion the Cornell Net Carbohydrate and Protein System (CNCPS) will be utilized to describe the modeling aspects related to ME, MP and amino acid supply and requirements (Tylutki et al., 2008; Higgs, 2014; Van Amburgh et al., 2015). There are at least five major steps necessary to improve the prediction of MP and AA supply and requirements in a lactating and dry cow. Most of this discussion will involve basic structural changes in thinking relative to predictions and requirements. Those five areas are the use, characterization and application of crude protein, recycled urea and endogenous protein, intestinal digestibility and determining first limiting nutrients through integration of protein requirements with energy supply.

Crude protein

Crude protein is an antiquated metric and one that is difficult to apply to ruminants in general. A previous review and meta-analysis by Ipharaguarra and Clark (2005) clearly point out the inadequacy of using CP to describe milk yield. The first concern with CP is that it is a ratio of $(N \times 6.25)/DM$ which makes it difficult to partition into the amount of “CP” required to meet the ruminal requirements (Rumen degradable protein) for fermentation, microbial digestion and yield, and the amount of CP that escapes fermentation (rumen undegradable protein), especially if you are attempting to predict the AA supply to the cow. Further, as pointed out by Jones (1941) not all protein uses the same correction factor and this becomes more problematic as you attempt to describe the degradation of plant material through the gastrointestinal tract as the N content and thus the protein content changes (Van Soest, 1994). This, coupled with the ubiquitous use of 6.25 for all feeds analyzed and used in feed libraries creates variation in predicting individual MPAA supplies to the cow. Overall, to better predict MP, there has to be consideration for the AA within the MP from feed, endogenous protein and microbes as that is what the cow requires for milk yield and productivity. Some of the current approaches commonly used might confound this approach.

Using soybean meal as an example, starting with 3,000 g of soybean meal which is 51.5% CP or 8.24% N and 1.34% methionine on a CP basis or 0.64% methionine on a DM basis the grams of content can be calculated either way. In Table 1, the left column uses a typical approach of methionine as a percent CP which results in 20.7 g of methionine in the soybean meal whereas on the right side the calculation was made on a DM and N basis resulting in 19.2 g methionine, a difference of 1.5 g. Although this difference looks modest, when arrayed across all of the feed and other sources of AA leads to significant differences, especially when formulations attempting to reconcile diets with grams.

Table 1. Grams of methionine in soybean meal calculated as a percent of crude protein or on a nitrogen basis.

Grams CP	$3,000 \times 0.515 = 1,545 \text{ g}$	Grams N	$3000 \times 0.0824 = 247.2$
Met., %CP	1.34	Met. N, %DM	$0.64/0.0824 = 7.77\%$
Met., g	$(1,545 \text{ g} \times 0.0134) = 20.7 \text{ g}$	Met., g	$247.2 \times 0.0777 = 19.2$
		Or g x Met DM%	$3000 \times 0.0064 = 19.2$

Rumen ammonia, recycled urea and endogenous protein

An important factor that strongly influences rumen NH_3 -N is intake pattern, which in turn, impacts microbial growth. A comparison of predicted NH_3 -N using continuous intake, 4 meals/d and 8 meals/d is found in Figure 1. Microbial growth in the model becomes limited when rumen NH_3 -N falls below 5.0 mg/dl (Satter and Roffler, 1975). This interaction causes the behavior observed in Figure 2, where NH_3 -N falls below 5.0 mg/dl when the meal pattern is 4 meal/d. The effect of N recycling within the model is evident as rumen NH_3 -N slowly increases until the next meal is consumed. The same general pattern is presented by Schwab et al. (2005) using in-vivo

data. With continuous feeding and with 8 meal/d rumen $\text{NH}_3\text{-N}$ remains above 5.0 mg/dl, demonstrating the importance of feeding pattern on rumen N supply. This suggests that improper time budgets and inadequate feed availability will negatively affect rumen N balance. In response, diet N content would need to be increased to keep rumen ammonia above the desired level for adequate microbial function.

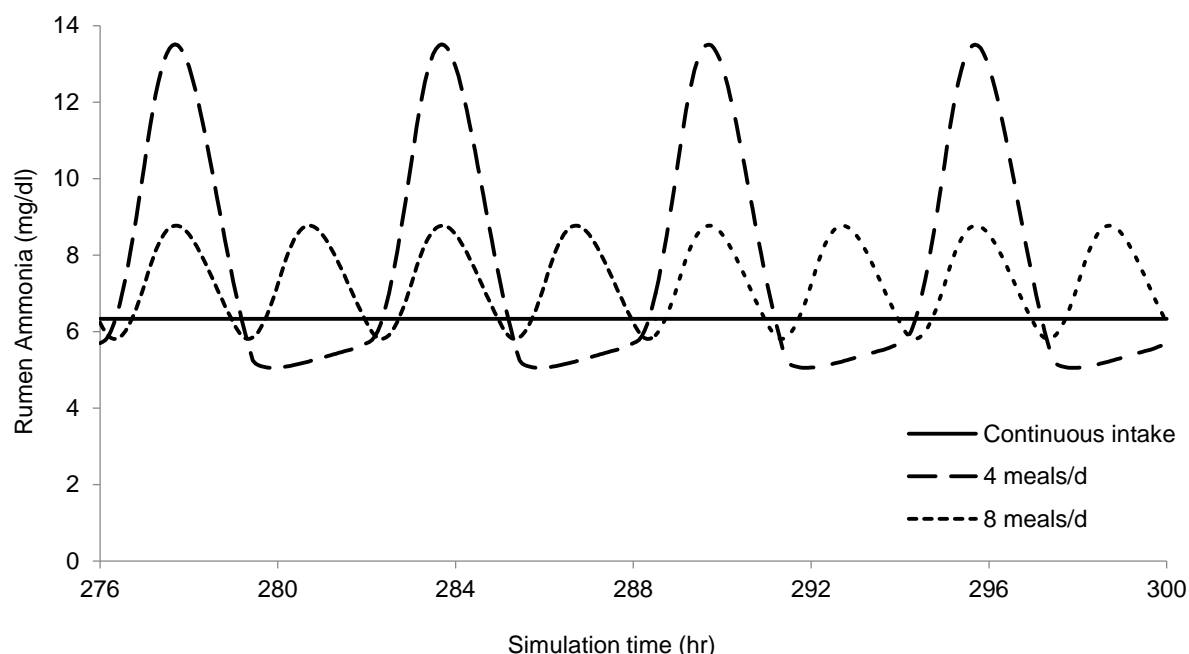


Figure 1. Variation in rumen $\text{NH}_3\text{-N}$ (mg/dl) among three different meal distributions represented by continuous intake, four meals per day and eight meals per day. The ammonia concentration does not decrease below 5 mg/dl in this example because below that value, microbial yield is decreased thus offsetting the demand.

Ruminants have a remarkable ability to recycle N back to the GIT in order to sustain favorable conditions for microbial protein synthesis. This recycling appears to be an obligate function with a low energy requirement (Reynolds, 1992). While previous versions of the model have accounted for N recycling (Fox et al., 2004), the dynamics are difficult to capture in a static model. A great deal of work has taken place to try and understand the exact mechanisms and quantitative aspects of N recycling, and while the exact mechanisms remain evasive, quantitative aspects of N fluxes are reasonably well understood and described (Lapierre and Lobley, 2001; Marini et al., 2008; Marini and Van Amburgh, 2003; Recktenwald et al., 2014).

The proportion of urea returned to the GIT relative to urea production is remarkably uniform among experiments when animals are fed diets at, or in moderate excess of MP requirements (Lapierre et al., 2004, Ouellet et al., 2004, Recktenwald, 2007, Valkeners et al., 2007). However, recycling increases when N supply is limited (Reynolds and Kristensen, 2008, Valkeners et al., 2007) and decreases when N supply is greatly in excess (Lapierre et al., 2004, Reynolds and Kristensen, 2008). To estimate the proportion of urea returned to the GIT in v7 of CNCPS, the equations presented in Recktenwald et al. (2014) and Reynolds and Kristensen (2008) were used

in combination. Recktenwald et al. (2014) showed a linear relationship between urea production and urea recycling in high producing cows fed diets ranging from 15% - 17% CP, while, Reynolds and Kristensen (2008) showed an increase in the proportion of urea recycled at very low N intakes. Therefore, using equations from Recktenwald et al. and Reynolds and Kristensen in combination allowed for a wider range in dietary conditions to be represented. Recycled urea is distributed to either the rumen, large intestine or small intestine and continues to cycle through the system at steady state. Overall, between 50 and 70% of intake N is converted to urea N and approximately 50% of the urea production is recycled into the GIT. The total pool size of N in the animal dictates how much of the hepatic urea production is captured in the rumen or excreted in the urine. As N intake increases, the probability of recycled urea N being captured by the microbes is reduced; thus, the objective during formulation is to find the balance between the ruminal requirements for ammonia N, N intake and the capacity for N recycling to ensure the optimum efficiency of use.

Urea N is not the only recycled N source in cattle. The endogenous nitrogen (EN) secretions occur at various places along the gastrointestinal tract (GIT). Important sources include saliva, gastric juices, bile, pancreatic secretions, sloughed epithelial cells and mucin (Tamminga et al., 1995). Gross EN to the forestomach and intestines were estimated in the CNCPS according to Ouellet et al. (2002) and Ouellet et al. (2010), which were subsequently partitioned into individual components (Table 1) using estimates reported in Egan et al. (1984). Endogenous contributions are reasonably consistent among diets when expressed relative to DMI or OMI (Marini et al., 2008; Ouellet et al., 2010; Ouellet et al., 2002; Tamminga et al., 1995). Thus, the model expresses each component as g EN per kg DMI. A summary of the EN contributions to various points in the GIT are in Table 2.

Table 2. Endogenous contributions used to predict endogenous AA requirements and supply in v7 of the CNCPS.

Endogenous component	Secretion (g N/kg DMI)
Saliva	0.9
Rumen sloughed cells	4.3
Omasum/abomasum sloughed cells	0.3
Omasum/abomasum secretions	0.2
Pancreatic secretions	0.4
Bile	0.1
Small intestine sloughed cells ¹	0.7
Small intestine secretions ¹	0.7
Large intestine sloughed cells	0.3

¹ Includes secretions past the pancreatic and bile duct and prior to the terminal ileum

Intestinal digestibility

Current cattle diet formulation models rely on library estimates of intestinal digestibility of proteins and carbohydrates to predict metabolizable energy (ME) and protein (MP) supply

(NRC, 2001; Fox et al., 2004; Tylutki et al., 2008). As models become more accurate and precise in the prediction of nutrient supply and nutrient balance, there is a greater need to evaluate and be able to adapt the inputs currently used as static library values. As diets are formulated closer to the MP requirements of cattle and subsequently lower in N content, accurate estimates of intestinal digestibility (ID) or indigestibility of protein and amino acids are increasingly important to ensure an adequate supply of those nutrients. Use of outdated feed library values for all feeding conditions can lead to under- and over-estimations of MP and amino acid supply, resulting in variation from expected production.

Several approaches have been developed to predict the intestinal digestibility of protein in feeds and are a departure from the detergent system of feed chemical composition (Calsamiglia and Stern, 1995; Ross et al., 2013). An in vitro assay was developed by Ross et al., (2013) that predicts intestinal N indigestibility in cattle using a multi-step approach. A study was conducted by formulating two different diets in high producing cattle using two different blood meals with different predicted intestinal protein indigestibility to test the accuracy and precision of both the assay and our ability to apply those values in the CNCPS for diet formulation.

Ninety-six multiparous cows (726 ± 14.2 kg BW; 147 ± 64 DIM) and thirty-two primiparous cows ($607 \text{ kg} \pm 29.5$ kg BW; 97 ± 20 DIM) were distributed by DIM and BW into 8 pens of 16 cows (12 multiparous and 4 primiparous). Pens were stratified into four levels of milk production, and each stratum randomly allocated to treatments. Diets were formulated using CNCPS, (Van Amburgh et al., 2015) using the chemical composition of the ingredients used in the experimental diets. The lactation trial consisted of a two week adaptation period, one week covariate period and 9 week experimental period at Cornell University Ruminant Center (Harford, NY). All cows were fed the LOW uN diet during adaptation and covariate periods. Cows were housed in pens under a four row barn design with one bed and more than one headlock per cow and free access to water. All cows received rBST (Posilac, Elanco Animal Health, Indianapolis, IN) on a 14 day schedule throughout the length of the trial.

Overall DMI and N intake for the treatments were similar and milk yield was significantly different for cattle fed the two treatments (Table 3). Milk yield was 1.6 kg/d lower for cattle fed the HIGH uN diet and energy corrected milk (ECM) was 1.9 kg/d lower on the same diet. Further, cattle fed the HIGH uN diet had significantly lower MUN levels than cattle fed the LOW uN diet (Table 4). From this information, it is apparent that the cattle fed the different blood meals had significantly different MP supply, consistent with the predicted values from the uN assay. The predicted difference described earlier (38.5 g N) is equal to approximately 240 g MP, about the amount required to produce 5 kg of milk under the conditions of this study.

However, the observed difference on an ECM basis was 1.9 kg, thus the difference between the absolute levels measured in the assay and the observed ECM yield are either due to differences in digestibility within the cow, the amount of the blood meal arriving at the small intestine, the amount of nutrients partitioned to body reserves, or a combination of all of those factors. Although the change in BW and BCS were not significant, the changes are still biologically relevant given the partitioning of nutrients to reserves and away from milk.

Table 3. The ingredient content and chemical composition of two diets containing blood meals with Low and High indigestible intestinal N digestibility.

Ingredient, % DM	Treatment	
	LOW uN	HIGH uN
Alfalfa haylage	11.5	11.5
BMR corn silage	49.3	49.3
Bakery	1.8	1.8
Blood meal High	3.7	---
Blood meal Low	---	4.0
Canola meal	3.0	3.0
Corn grain	16.1	16.1
Energy Booster 100	1.8	1.8
Molasses	1.8	1.8
Smartamine M	0.1	0.1
Sodium bicarbonate	0.6	0.5
Soybean hulls	4.6	4.5
Urea	0.2	0.2
Wheat midds	4.6	4.5
Min/vit mix	1.0	1.0
Chemical composition		
DM, % as fed	50.00	50.50
CP, % DM	15.20	15.20
NDF, % DM	31.90	32.30
ADF, % DM	21.30	20.50
Ether extract, % DM	4.30	3.90
Starch, % DM	30.40	31.20
Sugar, % DM	3.60	3.30
Ca, % DM	0.65	0.60
P, % DM	0.43	0.43
ME ¹ , Mcal/kg DM	1.80	1.70
Lys:Met ¹ , % MP	3.21	3.19

LOW: low uN diet, HIGH: high uN diet. ¹CNCPS predicted

To evaluate the outcome of the study, CNCPS v6.5 with the updated feed library rates and pool sizes was used to evaluate the predictions. The chemical composition of the feeds used in the study was inputted into the model. To evaluate the assay within the structure of the model and against the study data, the blood meal values for the uN and ADIN were the only values changed. For the two blood meals, the uN values were inputted in place of the ADIN value, and intestinal digestibility left at zero. Further, the intestinal digestibility of the NDIN value were set to 100% although after being analyzed for aNDFom, the blood meals do not contain any ND residue, so that pool is zero. With this approach, all of the protein in blood meals is in the A2, B1 and C fractions.

The current intestinal digestibility of the NDIN fraction for all feeds is 80% and it appears that the assay of Ross et al. (2013) captures that portion of the indigestible protein, therefore by difference; the remaining fractions should be set at 100% digestibility. Thus, with continued testing and implementation of the uN assay for all feeds, the NDIN fraction ID will be set to 100% because it appears that in NDF containing feeds, the uN assay spans both the ADIN and NDIN fractions.

Table 4. Effect of N availability on intake, milk production, milk composition and body weight gain of dairy cows fed diets with low and high unavailable N

Item ¹	Treatment		SEM	P-value
	LOW uN	HIGH uN		
DMI, kg	27.40	27.10	0.61	0.75
N Intake, kg DM	671.10	664.40	14.80	0.77
<u>Milk production</u>				
Milk, kg	42.00	40.40	0.31	<0.01
ECM, kg	41.90	40.00	0.32	<0.01
Fat, kg	1.51	1.42	0.02	<0.01
Protein, kg	1.26	1.23	0.01	0.03
<u>Milk composition</u>				
Fat, %	3.60	3.50	0.03	<0.03
Protein, %	3.03	3.06	0.02	0.20
Lactose, %	4.90	4.86	0.02	0.18
MUN, mg/dl	9.40	8.00	0.18	<0.01
SCC (log1000/ml)	3.90	4.00	0.05	0.13
<u>BW and BCS</u>				
BWinitial, kg	684.10	692.10	10.10	0.58
BWchange, kg	34.70	29.70	2.25	0.12
BCSchange, (1-5)	0.20	0.16	0.03	0.29
<u>Efficiency</u>				
Feed efficiency ²	1.56	1.50	0.03	0.34
Milk N efficiency ³	30.00	29.70	0.70	0.76

¹ DMI: dry matter intake, ECM: energy corrected milk yield (Tyrrell and Reid, 1965)

² calculated as kg milk / kg DMI

³ calculated as milk N/N intake*100

For the cattle inputs, the expected BW change based on the target growth approach was used and the BCS change was also inputted over the period of the study (9 wks), which accounted for the distribution of nutrients to other productive uses and not just milk output. With all of the inputs accounted for, the prediction of ME and MP allowable milk with the uN assay information is in Table 3.

In the CNCPS evaluation in Table 5, it is apparent that the feed chemistry described through the detergent system is not appropriate to allow the model to predict the most limiting nutrient in this comparison using blood meal as the treatment. When the uN data are used to describe the chemistry of the blood meals, the model provides an acceptable and realistic prediction of the most limiting nutrient. It is also important to recognize that an accurate and complete description of the animal characteristics was crucial to make this evaluation and in the absence of that information, the model would predict over 4 kg of MP allowable milk difference. The sensitivity of the model predictions to complete and accurate animal characterization cannot be overstated and helps explain why literature data to evaluate the model rarely allows for robust predictions of most limiting nutrients due the lack of complete information.

Table 5. The actual and energy corrected milk and the metabolizable energy (ME) and protein (MP) allowable milk for both treatments predicted by the CNCPS using the assay data of Ross et al., (2013) to estimate intestinal digestibility of blood meal, or using the original fractionation approach using acid detergent insoluble nitrogen as the unavailable fraction

Item	Treatment	
	LOW uN	HIGH uN
Actual milk, kg	42.0	40.4
Energy corrected milk, kg	41.9	40.0
<i>Using uN assay inputs</i>		
ME allowable milk, kg	45.0	46.0
MP allowable milk, kg	42.6	39.3
<i>Using NDIN and ADIN</i>		
MP allowable milk, kg	44.9	44.6

In summary, the uN assay appears to provide protein indigestibility predictions that are consistent with cattle responses and serves as a platform for modifying the approach to predict protein digestibility within the CNCPS and will improve the model's ability to identify the most limiting nutrient. The data also demonstrate that we are ready to move beyond the detergent system of fractionation for protein, to a system that fractionates proteins based on solubility and indigestibility.

Accurate and complete amino acid measure and values

Most systems use a factorial approach to calculate AA supply, so accurate profiles of AA in undegraded feed, bacteria, protozoa, and endogenous portions of post-rumen protein flows are important. The AA content of protein has historically been determined by single time point hydrolysis, as this represents a compromise between maximal release of AA from the matrix while minimizing the loss of acid labile AA (Rutherford, 2009). Determination at multiple time points followed by least-squares non-linear regression provides more accurate estimates of the true amino acid profile (Darragh and Moughan, 2005).

Microbial samples obtained from the omasum were used to determine the AA content after multiple time point hydrolysis. The AA content of all samples was determined by HPLC

following hydrolysis at 110°C in a block heater (Gehrke et al., 1985) for 2, 4, 6, 12, 18, 21, 24, 30, 48, 72, 120 and 168 h. All AA except Trp were determined using 6N HCl hydrolysis, with Met and Cys undergoing an additional pre-oxidation step. Tryptophan was determined using fluorescence detection after hydrolysis in barium hydroxide at the same time points as the acid hydrolysis. The entire time course was performed twice for each sample, and the reported values are the mean of the two determinations.

Table 6. Comparison of measured AA composition after single hydrolysis time point vs. estimated AA composition determined using least-squares non-linear regression after multiple hydrolysis times for omasal bacteria and protozoa isolates from trial B.

Item	Bacteria			Protozoa		
	24 h ¹	Mult ²	% Δ	24 h ¹	Mult ²	% Δ
Essential AA, % of AA						
ARG	4.96	4.88	1.6	5.37	5.41	-0.7
HIS	2.24	2.17	3.0	2.50	2.59	-3.6
ILE	4.25	4.77	-12.4	4.03	4.51	-12.0
LEU	5.48	5.47	0.3	6.83	6.43	5.8
LYS	7.52	7.40	1.6	8.90	8.79	1.2
MET	4.71	4.81	-2.0	3.44	3.87	-12.6
PHE	6.15	5.94	3.4	6.79	6.76	0.4
TRP	5.51	5.93	-7.7	4.26	5.49	-29.1
THR	5.67	5.70	-0.5	4.84	5.09	-5.1
VAL	6.58	7.14	-8.4	4.67	4.88	-4.6
Total EAA	53.07	51.73	2.5	51.61	51.01	1.2
Non-essential AA, % of AA						
ALA	6.68	7.15	-7.0	5.36	5.17	3.6
ASP	10.46	11.13	-6.3	9.65	10.42	-7.9
CYS	1.43	1.45	-1.4	2.37	2.22	6.5
GLU	11.25	11.39	-1.3	12.94	13.40	-3.5
GLY	5.01	4.98	0.6	4.67	4.53	2.9
PRO	2.00	1.97	1.2	2.99	2.97	0.7
SER	4.48	5.03	-12.2	5.14	5.43	-5.8
TYR	5.61	5.82	-3.6	5.27	4.83	8.3
Total NEAA	46.93	48.90	-4.2	48.39	49.22	-1.7
Total AA, % of DM	346.60	339.00	2.2	295.00	290.70	1.4

¹AA composition after 24 h hydrolysis time

²AA composition determined from least-squares non-linear regression from multiple hydrolysis times.

The comparison of the multiple time point vs. single time point indicates that the AA profile is affected by the rate at which AA are hydrolyzed in the assay. This means that when using a single time point hydrolysis at 21 or 24 h, the acid labile and slower releasing AA will be

underestimated, while the faster releasing and acid stable AA would be overestimated. In a quantitative sense, this might not account for much of the rumen-undegraded portion individual feed ingredient AA. However, when assigning a profile of AA to the microbial flows, error in the analysis will have a large effect on predicted AA flows when using the factorial approach, as the microbial portion is usually responsible for 40-60 % of the total AA supply.

Similar measurements have been made for feeds and similar differences have been found, especially in the branched chain amino acids (Table 7). This data suggests that the 21 hr hydrolysis of AA (Gehrke et al., 1985) does not completely recover all of the AA. Further, a multiple time point process should be evaluated to determine if the release of more slowly hydrolyzed AA are uniformly recovered after the 168 hr hydrolysis or if more feeds need to be analyzed to determine the true AA content.

Table 7. The amino acid composition (mg/g DM) of three feeds analyzed at 24 and 168 hr of hydrolysis and content calculated by logistic regression of the content of the residues.

	Canola 1			Soybean meal 1			Blood meal		
AA ¹	24h	168h	SEM ²	24h	168h	SEM ²	24h	168h	SEM ²
Arg	22.17	20.84	0.67	30.99	34.74	1.88	36.26	39.20	1.47
His	12.50	11.04	0.73	12.40	13.95	0.78	50.40 ^a	61.58 ^b	5.59
Ile	12.40 ^a	14.27 ^b	0.93	18.99	21.07	1.04	6.06	6.19	0.06
Leu	22.48	25.53	1.53	30.26	39.19	4.47	96.90 ^a	119.97 ^b	11.54
Lys	17.76 ^a	19.54 ^b	0.89	25.39	31.70	3.16	71.77 ^a	87.10 ^b	7.67
Phe	18.51	17.81	0.35	25.88	29.09	1.61	67.93	71.90	1.99
Thr	15.08 ^a	12.31 ^b	1.39	17.30	14.92	1.19	37.75	37.64	0.05
Val	17.22	18.40	0.59	20.65	21.91	0.63	59.52 ^a	83.09 ^b	11.79
Met	10.81	11.55	0.37	9.10 ^a	9.78 ^b	0.34	17.56	17.50	0.03
Trp	8.82	9.42	0.30	10.57	10.48	0.05	25.14 ^a	56.32 ^b	15.59

^{a, b} Different superscripts for a given feed at 24h vs 168h signifies $p < 0.05$

Concept of first limiting nutrient and integration of ME and AA

Requirements for each individual EAA in the CNCPS are predicted for processes that are quantified by the model (maintenance, lactation, pregnancy, growth) and subsequently divided by the efficiency of transfer to that process to give the total AA requirement (O'Connor et al., 1993; Fox et al., 2004). The efficiency of transfer could also be thought of as the additional requirement for each AA relative to the requirements quantified by the model. Such processes include oxidation across the gut or in other tissues, anaplerotic requirements, synthesis of non-essential AA, gluconeogenesis etc. (Lapierre et al., 2005; Lapierre et al., 2006; Lemosquet et al., 2010; Lobley, 2007). The apparent efficiency of AA use for any given diet can be calculated by dividing model predicted amino acid requirement (AAR) by amino acid supply (AAS), which can be variable, and typically decreases as AAS increases relative to either AAR or

metabolizable energy (Hanigan et al., 1998). This decrease in apparent efficiency of AA use represents AA being increasingly used for purposes other than those quantified or described by the model. If the utilization of each AA for every process in metabolism could be adequately quantified, the term 'efficiency of use' would become obsolete as it would be 100% (there would be no additional requirement above model predictions). The ability of cows to direct AA to other uses demonstrates the interactions among different nutrients and is an example of the metabolic flexibility that allows productivity to be maintained across a wide range of nutrient inputs and supply (Lobley, 2007). The pertinent question for ration balancing is: what level of additional AA supply is required above the predicted requirements for milk protein synthesis and body protein requirements to maximize productivity and minimize AA wastage? The answer to this question is going to differ among models as supply and requirements are calculated in different ways.

The optimum supply of EAA in v7 was estimated similarly to Doepel et al. (2004) using a dataset of studies that infused AA into the abomasum, duodenum, or intravenously and fitted a logistic curve (Higgs, 2014). The optimum supply of each EAA was defined as the point in which a logistic curve was approaching plateau most rapidly (Lysine example; Figure 2). This point is similar to the break-point in the segmented linear model used in the NRC (2001). The optimum ratio of model predicted AAR to AAS (efficiency of use) for each AA in v7 are in Table 8. The impact of energy supply on the utilization of AA was also investigated by regressing the ratio of AAR and AAS against AA supply relative to total ME (Lysine example; Figure 3). Interestingly, the optimum supply of Met and Lys estimated using this approach was 15.1% and 5.7% of EAA, respectively, which is similar to results found in other studies that used different approaches (Rulquin et al., 1993; Schwab, 1996; Schwab et al., 1992). However, under these circumstances, no relationship was observed between the 'efficiency' of AA use when AA supply was expressed relative to MP supply but a strong relationship was observed when AA were expressed relative to ME supply which is in agreement the findings of Van Straalen et al. (1994). These data suggest when balancing rations it might be more appropriate to consider AA supply relative to ME which is the approach used in swine (NRC, 2012). Establishing requirements for monogastrics is less complicated than in ruminants as the true AA supply is more easily determined (Lapierre et al., 2006). That being said, with the available AA infusion study data and the updated techniques described previously in this paper, AA requirements in the ruminant animal are becoming both more accurate and precise. To extend the comparison of non-ruminant to ruminant, the predicted Lys requirement for a lactating sow in the NRC (2012) model is 2.72 g Lys/Mcal ME which is similar to the 3.03 g Lys/Mcal ME calculated in this study for dairy cows. Likewise, the recommended ratios for each EAA and Lys are similar in the dairy cow and sow with the exception of Met and His (Table 8). These data suggest, as improvements are made to the predictions of true AA supply in dairy cows, consideration of the approach used to balance AA in other species where AA supply is more easily determined could provide opportunities to improve productivity and the efficiency of nutrient use.

Summary

To better describe AA supply and requirements and develop approaches to formulate closer to meet the requirements, several steps have been taken to improve the predictions. These

approaches provide solutions to offset bias in calculations, improve chemistry to provide information about improved recoveries and digestibilities and provide new insights into how to evaluate AA requirements on an energy allowable basis consistent with monogastric species. It is anticipated that actualizing all of these approaches will allow for lower N feeding and more efficient diets that result in lower cost and less environmental impact of dairy cattle.

Table 8. Efficiency of use and optimum supply of each EAA relative to total EAA, ME and Lys.

AA	Efficiency of use	% EAA	g AA/ Mcal ME	Lys:AA Dairy ¹	Lys:AA Swine ²
Arg	0.55	10.2%	2.04	1.49	1.85
His	0.70	4.5%	0.91	3.33	2.50
Ile	0.61	10.8%	2.16	1.40	1.78
Leu	0.67	17.1%	3.42	0.89	0.89
Lys	0.62	15.1%	3.03	1.00	1.00
Met	0.53	5.7%	1.14	2.66	3.71
Phe	0.53	10.7%	2.15	1.40	1.82
Thr	0.53	10.7%	2.14	1.41	1.49
Trp	0.58	2.9%	0.59	5.16	5.33
Val	0.62	12.4%	2.48	1.22	1.15

¹ Optimum Lys:EAA ratio for the data set used

² Optimum Lys:EAA ratio for a lactating sow (NRC, 2012)

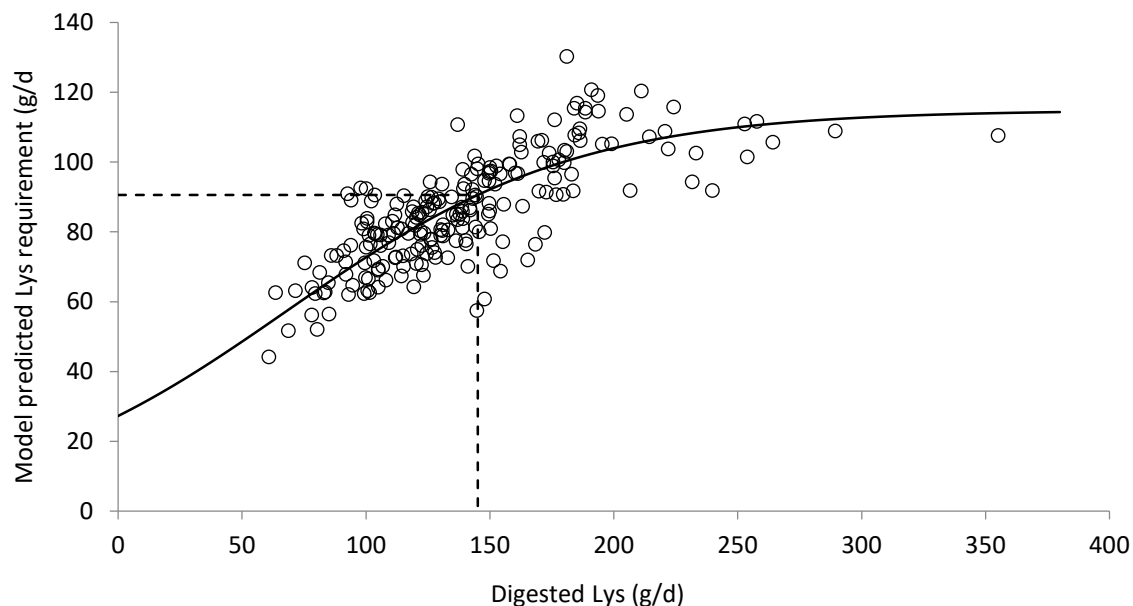


Figure 2. Logistic fit of model predicted Lys requirement and Lys supply. The dashed line represents the optimum ratio of Lys requirement and Lys supply

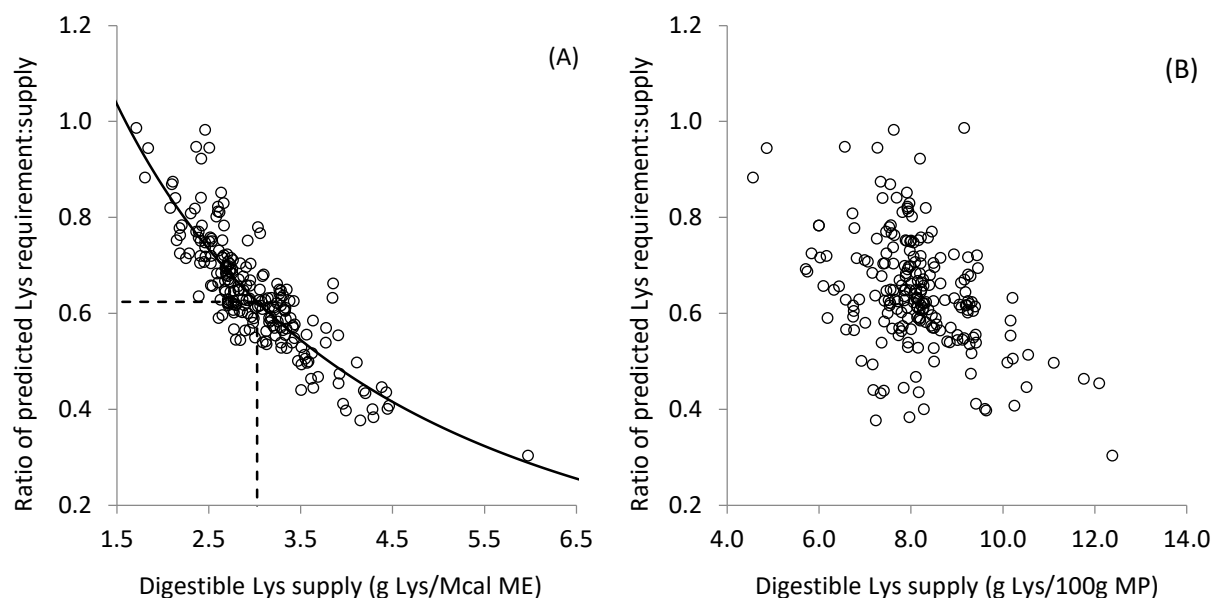


Figure 3. Relationship between model predicted Lys requirement:supply and Lys supply relative to ME (A) or MP (B). The dashed line in (A) represents the Lys supply at the optimum ratio of model predicted Lys requirement and supply. No significant relationship was determined in (B).

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Advances in the Understanding of Dietary Fibre and its Components in Relation to the Use of Alternative Feed Ingredients in Modern Poultry and Livestock Production

Progrès réalisés dans la compréhension de la fibre alimentaire et de ses composants dans le contexte de l'utilisation d'ingrédients alimentaires de remplacement en production animale aujourd'hui

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Abstract

Dietary fibre (DF) is defined as the “the remnants of plant cells resistant to hydrolysis by the digestive enzymes in the small intestine of non-ruminant species” and is composed of cellulose, hemicellulose, pectin and lignin. Other non-digestible substances, which may be included in a broader definition of fibre, are polyphenols (tannins), resistant starch, fructans, waxes, gums, oligosaccharides, cell wall glycoproteins and Maillard reaction products. Although the terms hemicellulose and pectin are still in use, describing these groups of polysaccharides as non-starch polysaccharides (NSP) would be more appropriate. With respect to analysis, the fibre fractions listed above are variously measured as CF, ADF, NDF, NSP, and total DF, with detergent methods providing a much better alternative to conventional CF. However, it should be noted that NDF values significantly underestimate DF due to losses of soluble NSP. Such losses account for 7% of DM and can only be taken into account following the measurements of total DF, which averages 16, 17, 33, 36, and 38% of DM for peas and faba beans, SBM, DDGS, canola meal, and lupin, respectively. Facts about DF in relation to the efficiency of animal production reflect the phrase “The Good, the Bad and the Ugly” with some NSP having positive effects on microbiota composition, SCFA production, control of enteric bacterial pathogens (prebiotic effect), and “gut health” when fed along with dietary enzyme supplements. Undoubtedly lignin is “the bad” fiber as it has a strong negative influence on fibre biodegradability. Products of final stages of Maillard reaction would definitely belong to “the ugly” category due to losses of available lysine, threonine, arginine, and reduced activity of digestive enzymes.

Résumé

La fibre alimentaire (FA) est définie comme « un vestige des cellules végétales résistantes à l'hydrolyse par les enzymes digestives dans le petit intestin des espèces non ruminantes » et est composée de cellulose, hémicellulose, pectine et lignine. D'autres substances non digestibles peuvent être incluses dans une définition plus large de la fibre : polyphénols (tannins), amidon résistant, fructosanes, cires, gommes, oligosaccharides, glycoprotéines de parois cellulaires et produits de la réaction de Maillard. Bien que les termes hémicellulose et pectine soient toujours

en usage, il serait plus juste de décrire ces groupes de polysaccharides comme des polysaccharides non amylacés (PNA). En ce qui concerne l'analyse, les fractions fibreuses énumérées précédemment sont exprimées de diverses façons – fibre brute, fibre ADF, fibre NDF, PNA et FA totale –, les méthodes au détergent offrant une solution nettement supérieure à la mesure de fibre brute conventionnelle. Il convient toutefois d'observer que les valeurs de NDF sous-estiment largement la FA en raison des pertes de PNA solubles. Ces pertes comptent pour 7 % de la MS et ne peuvent être prises en considération qu'après les mesures de FA totale, lesquelles atteignent en moyenne 16, 17, 33, 36 et 38 % de la MS pour le pois et la féverole, le tourteau de soya, la drêche de distillerie avec solubles, le tourteau de canola et le lupin, respectivement. Les faits révèlent du bon et du mauvais en ce qui concerne l'efficacité de la FA en production animale : en effet, certains PNA ont des effets positifs sur la composition du microbiote, la production d'AGCC, la maîtrise des agents pathogènes bactériens entériques (effet prébiotique) et la « santé du tube digestif » lorsqu'ils sont servis en combinaison avec des compléments alimentaires enzymatiques. Il ne fait aucun doute que la lignine appartient à la catégorie des mauvaises fibres, compte tenu de son effet négatif marqué sur la biodégradabilité de la fibre. Dans cette histoire, les produits des dernières phases de la réaction de Maillard ont aussi le mauvais rôle pour cause de pertes de lysine, thréonine et arginine disponibles et de diminution de l'activité des enzymes digestives.

Introduction

Dietary fibre is the most 'unknown' constituents of feed ingredients. It is confusing in terms of any physiological effects, unpredictable in facilitating the diet formulation, and most importantly it is the least understood component of feed ingredients. Fibre is a nutritional entity, not a chemical one. An understanding of the nutritional implications of dietary fibre has been hindered by confusion in the definition and thus analysis of this component. While a physiological definition of fibre as a dietary component resistant to mammalian digestive enzymes is now widely accepted, only the sum of dietary non-starch polysaccharides and lignin is often considered to represent the dietary fibre.

Several substances, non-digestible by endogenous enzymes, should be considered in a broader definition of the term fiber. These substances include condensed type of polyphenols (tannins), resistant starch, glycoproteins (i.e., cell wall- inserted protein, arabinogalactan protein), advanced glycation end products of Maillard reaction, or even minerals associated with the fiber components.

The objectives of this presentation are to review the current advances in the understanding of dietary fibre composition, content, the key nutritional and physiological effects of fibre components, and the crucial concepts of dietary fibre analysis. The properties and any antinutritional effects of different fibre components of cereal grains, wheat by-products, canola meal, expelled canola, flaxseed, soybean, and pulses will be identified. As well, the effect of processing on Maillard reaction, protein damage, and fibre formation will be illustrated. Finally, the use of enzyme technology for releasing bioactive and prebiotic-type fibre polysaccharide hydrolysis products to stimulate the proliferation of beneficial bacteria and pathogens exclusion will be documented. This, in turn, should encourage the use of high-fibre ingredients in poultry

and swine diets to reduce feeding cost while maximizing performance - the strategies for those involved in modern, antibiotic-free poultry and swine production.

Dietary fibre definition

The term “dietary fibre” was first introduced by Hipsley in 1953 to cover the non-digestible constituents of plants that make up the plant cell wall, known to include cellulose, hemicellulose and lignin. This definition was broadened by Trowell et al. (1976) and has become the most widely accepted definition of dietary fibre as “The remnants of plant cells resistant to hydrolysis by the alimentary enzymes of man. It is composed of cellulose, hemicellulose, oligosaccharides, pectins, gums, mucilages, waxes and lignin” (McCleary et al., 2015).

The debate on the definition of dietary fiber was finalized in 2009 by the Codex Committee on Nutrition (i.e., Codex Alimentarius Commission) defining fiber as “Carbohydrate polymers with ten or more monomeric units, which are not hydrolyzed by the endogenous enzymes in the small intestine of humans and belong to the following categories: (1) Edible carbohydrate polymers naturally occurring in the food, (2) Carbohydrate polymers, which have been obtained from food raw material by physical, enzymatic or chemical means, and (3) Synthetic carbohydrate polymers which have been shown to have a physiological effect of benefit to health. The decision on whether to include carbohydrates from 3 to 9 monomeric units was left to the discretion of national authorities. Canada and China have chosen to include these polymers.

It is of interest to note, that the Codex definition is not that different from the definition originally proposed by Trowell et al. (1976). In addition to carbohydrate polymers, Codex definition allows for the inclusion of lignin and/or other compounds associated with polysaccharides in the plant cell walls. Also, the Codex definition includes carbohydrate polymers, which have been obtained from food raw material by physical, enzymatic, or chemical means and which have been shown to have a physiological effect of benefit to health (McCleary et al., 2015).

As early as in 1967, the sequential fibre solubilization methods have been developed by Van Soest and co-workers (Van Soest and Wine, 1967) and have become widely used by animal nutritionist for the measurement of cellulose, hemicellulose, pectin, and lignin contents of plant feedstuffs. Gravimetric methods for neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) have been proposed. Although based on insoluble fibre measurements, the methods included a wide variety of fibre components important for animal nutrition. In addition to some fibre components similar to those identified by Trowell and Codex definitions, the detergent procedures provided a good estimate of other nutritionally important components, including cell wall-inserted proteins, high-molecular weight condensed tannins, or Maillard reaction products.

Dietary fiber components

A range of NSP that are present in feed along with lignin built up the dietary fibre. Other non-digestible substances, which may be included in a broader definition of the term fibre, are polyphenols other than lignin (i.e., large condensed tannins), resistant starch, cell wall-inserted protein, mucilages, gums, advanced glycation end products of Maillard reaction, oligosaccharides, and inorganic constituents (Table 1).

Table 1. Components of dietary fibre.

Class	Type
Structural polysaccharides	NSP ¹ - cellulose, hemicellulose, pectins
Non-structural polysaccharides	Mucilages, gums, resistant starch, inulin
Structural non-carbohydrate	Lignin, polyphenols ²
Protein/carbohydrate	Cell wall protein, Maillard products
Oligosaccharides	Galacto- and fructooligosaccharides
Other	Cutin, waxes, minerals

¹ Non-starch polysaccharides

² Includes high-molecular weight condensed tannins

Non-starch polysaccharides: cellulose, hemicellulose, pectins, mucilages, gums

Non-starch polysaccharides can be divided into cellulose and non-cellulosic polysaccharides. The structure of cellulose is characterised by long chains of (1-4) β -linked glucosyl residues while hemicelluloses represent a distinct group of polysaccharides which are classified according to their chemical composition and structure. Thus, arabinan is a polymer composed of L-arabinose units, galactan of D-galactose units, mannans of D-mannose units, etc. Most hemicelluloses, however, do not occur as homopolysaccharides, i.e., based upon one sugar residue only, but rather as heteropolysaccharides made up of at least two to four different types of sugar residues, usually as short appendages linked to the main backbone chain (Fig. 1). In other words, they are branched structures (Aspinall, 1970).

The term ‘hemicellulose’ refers to those polysaccharide constituents of plant cell walls that are soluble in alkali. The term was first proposed because of the close association of these polysaccharides with cellulose in the plant cell wall and, at that time, their presumed role as precursors in cellulose synthesis. Although it is now well established that hemicelluloses are not involved in the biosynthesis of cellulose, the term describing this group of non-cellulosic polysaccharides is still retained.

Pectic substances have been considered to include diverse types of polysaccharide structures based on linked units of D-galacturonic acid, D-glucuronic acid, D-galactose, L-arabinose and L-rhamnose. Being similar to hemicelluloses, pectins are a family of highly branched polymers associated with cellulose and hemicelluloses and thought to be solubilized in water prior to removal of hemicelluloses soluble in the alkaline solutions (Theander et al., 1989). Based on the author’s experience, however, pectins within feed ingredients are quite different from those

found in fruits and fruit products and more closely resemble cellulose and non-cellulosic polysaccharides of low solubility in water. Although the terms hemicellulose and pectin are still in use, describing these fibre fractions as non-starch polysaccharides (NSP) would appear to be more appropriate.

Some structural families, especially the rhamnogalacturonans and highly branched arabinogalactans, can be extended to include several gums and mucilages (i.e., flax mucilage). It has been suggested that the formation of mucilages and gums involves the apposition of additional sugar residues to the outer chains of polysaccharides already present in the plants.

Cell wall-inserted proteins (glycoproteins)

The cell walls of a variety of plants contain a unique hydroxyproline-rich glycoprotein. Up to 20% of the amino acid residues of this structural protein is hydroxyproline, and the protein itself accounts for up to 10% of the plant cell wall. The remaining amino acids are serine, histidine, lysine, tyrosine and valine. Carbohydrate accounts for approximately 40-60% of the weight of all cell wall glycoprotein with arabinose and galactose being the major carbohydrate constituents (Showalter and Verner, 1989).

Lignin and polyphenols

Another component which seems to be particularly associated with the plant cell wall polysaccharides is lignin. Lignin is a polyphenolic macromolecule and the basic units of plant lignin are thought to be 3,5-dimethoxy-4-hydroxy-phenylpropane, 3-methoxy-4-hydroxy-phenylpropane and 4-hydroxy-phenylpropane. Many types of crosslinks are possible between these basic units making the overall structure of lignin very complicated. Undoubtedly, lignin has a strong, negative influence on the biodegradability of plant cell walls.

High-molecular weight condensed tannins represent another important component of dietary fibre. Condensed tannins (or proanthocyanidins) comprise a group of polyhydroxy-flavan-3-ol polymers linked by carbon-carbon bonds between flavanol subunits. Their multiple phenolic hydroxyl groups lead to the formation of complexes with protein, metal ions and with other macromolecules like polysaccharides (Schofield et al., 2001).

Cell wall architecture

The structural features of polysaccharides are somewhat oversimplified by omitting interpolymeric linkages in the cell walls. Cellulose fibres make up an important part of the framework of the cell wall. The fibrils contain about 120 cellulose molecules which are bound together by a matrix of other polymeric materials such as non-cellulosic polysaccharides, structural protein and lignin. The cell wall then can be compared to cases of reinforced concrete, in which the cellulose fibrils correspond to the steel rods and the matrix material to the concrete (Lehninger, 1984).

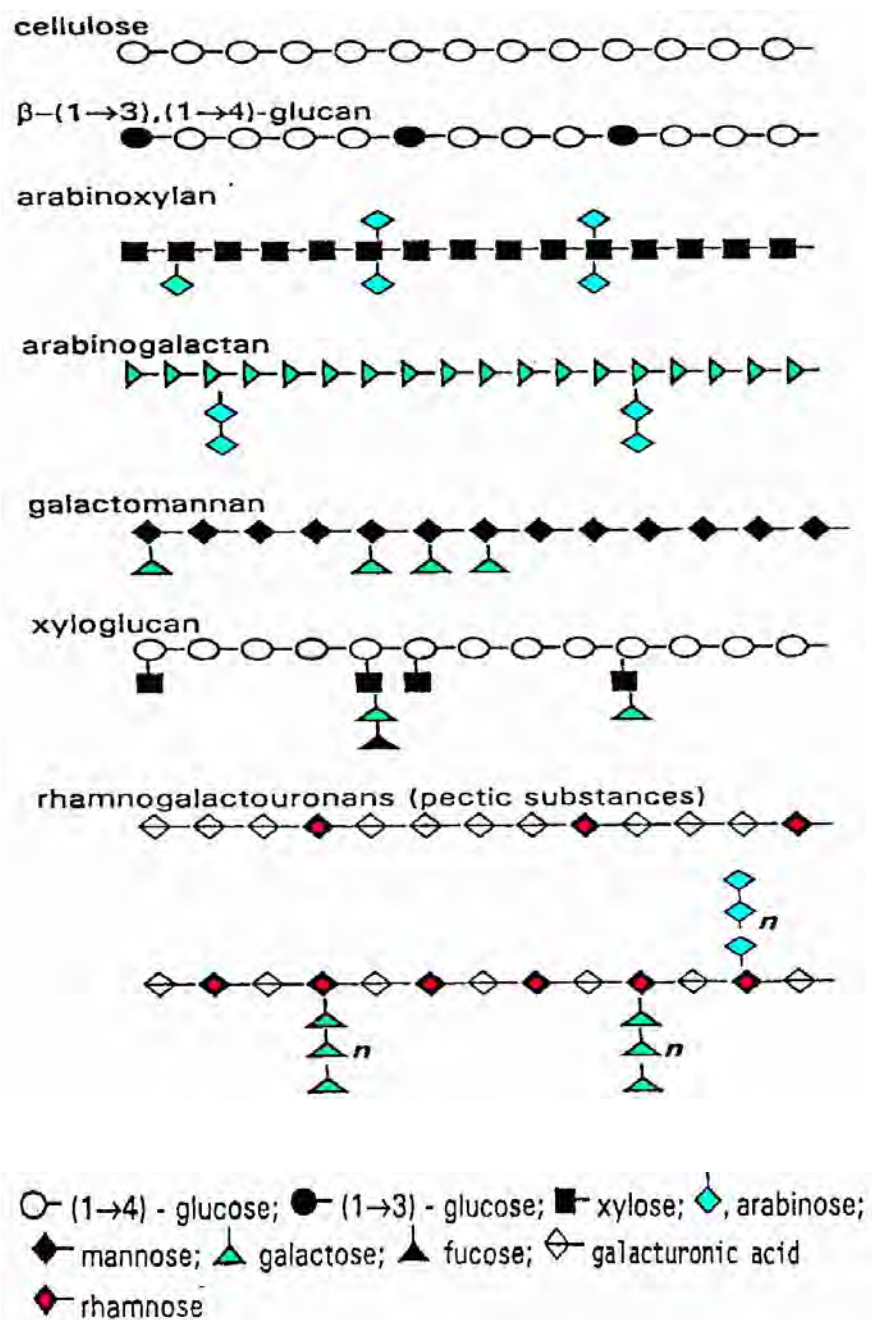


Fig. 1. Non-starch polysaccharide structures commonly found in feed ingredients of plant origin (Smits and Annison, 1996).

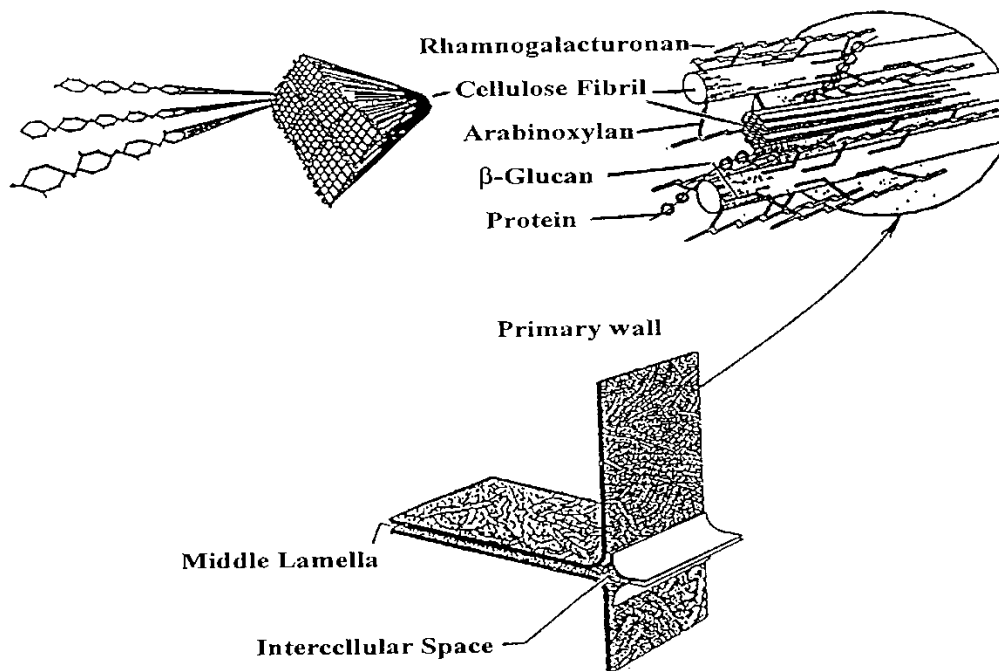


Fig. 2. A model of cell wall architecture (adapted from Albersheim, 1975, and Morchessault and Sundararajan, 1983).

Resistant starch

Another important component of dietary fibre is starch resistant to digestive enzymes. There are basically three types of resistant starch. Type I is starch physically trapped within the matrix of feed (i.e., starch granules locked in the plant cells) and is enzyme resistant simply because amylolytic enzymes may not have access to it. Type II represents native granular starch. Its enzyme resistance is ascribed to its compactness and partially crystalline structure and can, of course, be overcome by gelatinization. Type III is formed during retrogradation of starch during sequential cooking and cooling. Although it may well consist mainly of retrograded amylose, there are indications of increased enzyme resistance upon retrogradation of amylopectin. Type III resistant starch may act as dietary fibre and contribute to substrates available for microbial fermentation (Englyst et al., 1992). Currently, a variety of resistant starch products (i.e., cooked and cooled rice and potatoes, legumes, etc.) are recommended for human consumption as valuable sources of dietary fibre.

Advanced glycation end products of Maillard reaction

Another component(s) which should be considered in a broader definition of dietary fiber include advanced glycation end products. Maillard reaction or non-enzymatic heat catalyzed glycosylation, or glycation, is the covalent attachment of sugars to α - or ϵ -NH₂ groups of amino

acids and protein to form glycated proteins. The first glycation product - a glycosylamine or Schiff base rearranges to a more stable ketoamine or Amadori product. The Amadori products can then form cross-links with other amino groups. The resulting polymeric aggregates are called advanced glycation end products and are responsible for brown coloration and possibly aroma and flavor formation. In this final stage of the Maillard browning reactions, brown pigments (melanoidins) are formed through aldol condensation and polymerization of carbonyl compounds. The melanoidins formed in the amino acid-reducing sugar systems have a wide range of molecular weights (Friedman, 1996).

The advanced and final stages of the Maillard reaction contain a high degree of complexity. The chemistry of compounds formed in these stages is not well-known and their mechanisms are not well understood. In humans, advanced glycation end-products may play an important role in the pathogenesis of chronic diseases such as diabetes, atherosclerosis, renal failure, premature aging, and Alzheimer's disease (De Oliveira et al., 2016).

In general, only reducing sugars such as glucose, fructose, ribose, xylose, or arabinose react non-enzymatically with the amino groups of amino acids and protein and are more damaging to the nutritive value than the non-reducing sucrose or polysaccharide starch or cellulose. Losses in available lysine, arginine, threonine, aspartic acid, or glutamic acid due to early and advanced Maillard reactions are quite common. In addition, Maillard reaction products have an inhibiting effect on trypsin and intestinal brush border enzymes aminopeptidase and carboxypeptidase. The high-molecular weight fractions have been shown to inhibit the proliferation of gram-positive bacteria, such as *B. subtilis*, *Lactobacillus*, and *Staphylococcus* strains (Friedman, 1996).

Methods of dietary fibre analysis

The cell wall polysaccharides along with phenolic polymers and glycoproteins are variously measured as CF, ADF, NDF, NSP, and total DF in feed evaluation laboratories. The old CF method has been repeatedly abandoned, as it measures only a small and variable fraction of the total fibre. The detergent methods developed by Van Soest and co-workers provide a much more satisfactory alternative.

The detergent methods are inexpensive, fast, and with their automation by ANKOM Technology are more commonly used than the total dietary fibre (TDF) methods. However, the detergent methods show some deficiencies with the NDF values significantly underestimating the cell wall residues due to losses of water-soluble NSP. Such fractions not recovered in NDF include some legitimate cell wall components, such as arabinoxylans, β -glucans, as well as some storage polysaccharides, including mucilages and gums (Van Soest et al., 1991). As illustrated in Table 2, losses of EDTA/SDS-soluble NSP on NDF analysis would account for a significant portion of dietary fibre present in cereal grains, protein supplements, pulses, or forages.

Over the years, several enzymatic-gravimetric methods for TDF have been developed and are based on the digestion and removal of starch and protein followed by: 1) the measurement of the total indigestible residue as was the case for the AOAC methods 985.29 (Prosky TDF method) and 991.43; 2) the measurement of NSP and Klason lignin as in the Uppsala method; 3) the

measurement of NSP and resistant starch as in the UK method (Englyst method), or the measurement of NSP, lignin with associated polyphenols, and “fibrous” glycoproteins as is the case for the Manitoba method (Table 3).

Table 2. Loses of non-starch polysaccharides (NSP) on NDF analysis (10% moisture basis)

Feed ingredient	NDF	NDF-soluble NSP	Total dietary fibre
Cereal grains			
Corn	7.8	2.4	10.2
Wheat	9.2	3.1	12.3
Barley	15.9	7.8	22.7
Corn DDGS	29.4	2.6	32.0
Wheat DDGS	24.0	6.3	30.2
Protein supplements			
Soybean meal	12.2	7.1	19.3
Canola meal	27.0	8.0	35.0
Pulses			
Field peas	9.5	7.5	17.0
Faba beans	11.3	5.3	16.8
Forages			
Barley silage	42.6	8.7	51.3
Corn silage	31.1	5.0	36.1
Alfalfa	38.6	8.3	46.9
Grass	59.3	5.8	65.2
Alfalfa and grass mix	56.5	7.1	63.7

In the dietary fibre analysis by the Manitoba method (Slominski et al., 1994), the measurement of NSP is conducted on both the sample and the residue collected following NDF analysis and as such allows for the correction of substantial losses of detergent-soluble NSP. With additional analysis of the NDF residue, the procedure was extended to characterize and classify the fibre components into NSP, lignin with associated polyphenols, cell wall-inserted protein, and minerals associated with the fibre components. As the measurement of NSP by GLC involves quantitative analysis of each individual component sugar, it can also provide valuable qualitative data as to the type of polysaccharides present in the sample.

In line with the adapted definition of TDF by Codex Alimentarius, a new integrated TDF method was developed and validated by AOAC International (Methods 2009.01 and 2011.25). According to APAC, this is the only dietary methods that allow accurate measurement of carbohydrate-like dietary fibre components, including NSP, resistant starch, and nondigestible oligosaccharides (Table 3). From the animal nutrition point of view, however, the AOAC methods 985.29 and 2009.01 do not measure some important components of dietary fibre, notably cell wall protein, which along with advanced glycation end products of Maillard reaction, are effectively measured using the NDF procedure. Such protein-containing fibre fractions are often referred to as neutral detergent insoluble crude protein (NDICP).

Table 3. Total dietary fibre methods

Component measured	AOAC Gravimetric 985.29	Uppsala GLC ¹ -gravimetric	UK GLC	Manitoba Gravimetric-GLC	Codex/AOAC gravimetric-HPLC ² 2009.01
NSP ³	✓	✓	✓	✓	✓
Lignin/polyphenols	✓	✓		✓	✓
Resistant starch	✓		✓	✓	✓
Cell wall protein				✓	
Maillard products				✓	
Oligosaccharides				✓	✓

¹ Gas-liquid chromatography

² High-performance liquid chromatography

³ Includes cellulose, hemicellulose, pectins, mucilages, and gums

As indicated earlier, a significant underestimation of NSP and thus TDF content occurs when using the detergent methods, including NDF. Such deficiency, however, could be minimised by predicting the TDF values, with a reasonable degree of accuracy, from the respective measurements of NDF contents of any given feed ingredient (Fig. 2).

Another advantage of Van Soest detergent methods and that of the NDF-based Manitoba method is the measurement of condensed tannins (proanthocyanidins) which may be of importance due to their variable nutritional effects. In general, the growth depression caused by tannins is due to an adverse effect on protein and starch digestibility as tannins interfere with the digestive action of trypsin and alpha-amylase either by binding the enzymes themselves or by binding of the dietary protein into an indigestible form. However, such negative effects may be more related to tannins extracted with aqueous acetone or methanol. It is well recognised that some proanthocyanidins are insoluble in common solvents, including the acid-butanol system and, therefore, their content in plant materials may be underestimated. Tannins insoluble in most solvents may include higher molecular weight tannins and tannins bound to fiber (i.e., NDF-bound) (Giner-Chavez et al., 1997).

The butanol-HCl-iron method is widely used for measurement of extractable condensed tannins (proanthocyanidins) in foods and feeds. As the method is based on acid catalysed oxidative depolymerization of condensed tannins into anthocyanidins, this method has also been used for determination of bound condensed tannins. In the study by Harinder et al., (1999), however, an incomplete recovery of bound condensed tannins by the assay was demonstrated.

Brown-seeded (dark-flowered) cultivars of peas contain condensed tannins located in the seed coat while the yellow- and green-seeded cultivars are devoid of tannins (Igbasan et al., 1997). In agreement with canola, the brown-seeded, high-tannin characteristic of peas was reflected in its higher lignin and polyphenols (4.3 vs. 3.1%) and thus total dietary fiber (21.9 vs. 20.0%) contents when compared to yellow and green peas. Similar differences in ADF and NDF contents were observed for high- and low-tannin faba beans.

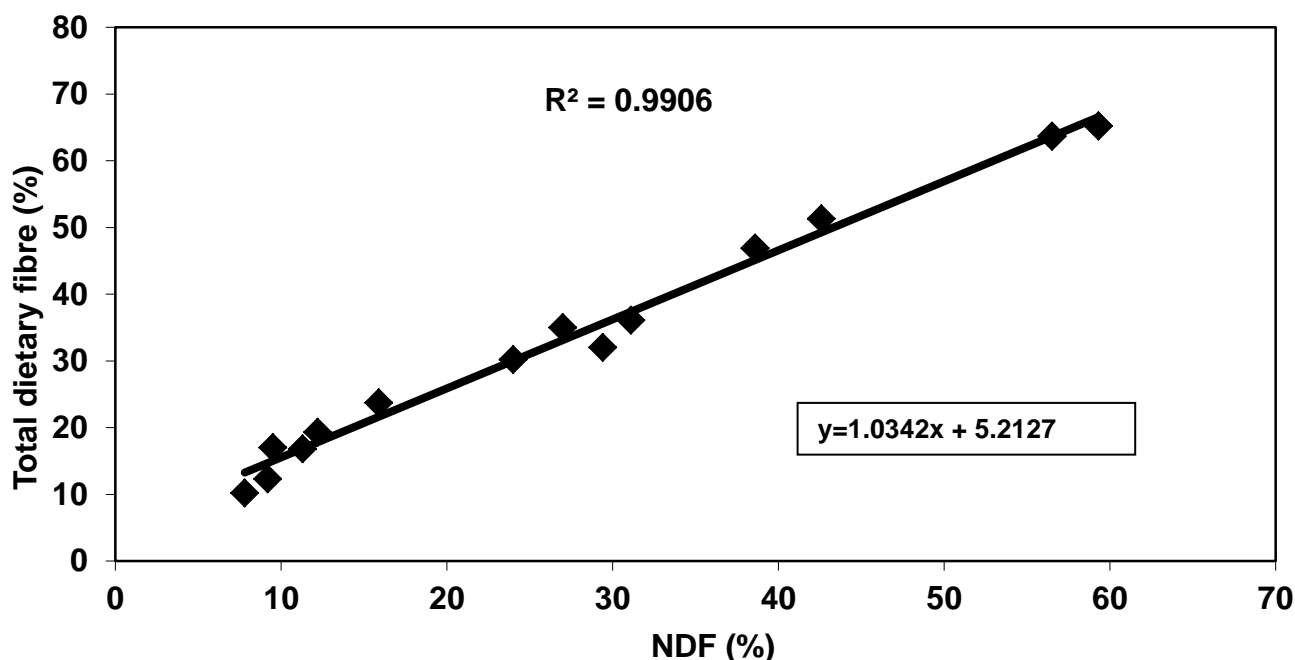


Fig. 2. Relationship between neutral detergent fibre (NDF) and total dietary fibre contents (10% moisture basis)

As illustrated in Table 4, ADF, NDF, and TDF methods effectively identified the differences in condensed tannin contents of different types of canola (black- or yellow-seeded), peas (brown or yellow-seeded) or faba beans (colored-flower high-tannin type or white-flower low-tannin type).

Table 4. Dietary fibre contents of low- and high-condensed tannin types of canola, peas, and faba beans (% DM).

Fibre fraction	Canola		Peas		Faba bean	
	Black	Yellow	Brown	Yellow	High-tannin	Low-tannin
Acid detergent fibre	20.1	9.3	-	-	12.9	11.0
Neutral detergent fibre	25.2	19.0	-	-	15.3	12.8
Total dietary fibre	35.0	29.8	21.9	20.0	-	-
Lignin and polyphenols	10.1	3.1	4.3	3.1	-	-

Bound proanthocyanidins seem to be inert and do not affect microbial fermentation in an in vitro rumen fermentation system. They are not hydrolysed by rumen microbes. However, these bound proanthocyanidins get released into the medium as a result of microbial action and then affect microbial fermentation as an increase in gas production of up to 100% was observed. In their study, NDF samples rich in bound PA did not decrease the cfu of *C. perfringens* type A strain known to be highly sensitive to tannins (Makkar et al., 1997).

Selection for yellow seed coat color, a visual marker of lower polyphenol (proanthocyanidins) content has been a major agronomic trait for Canadian canola improvement as it is linked to increased seed oil, protein and sucrose contents at the expense of fibre components (Slominski et al., 1999). It has been demonstrated that NDF and TDF content of yellow-seeded canola are lower than those of its black counterpart, primarily due to the lower lignin and polyphenol contents (Table 4). However, detailed evaluation involving available energy and amino acid measurements and growth performance of broiler chickens and turkeys revealed that breeding for low-fibre canola would result in quantitative rather than qualitative changes as evidenced by increased oil, protein, and sucrose contents and decreased fibre content in the seed. Among the fibre components, proanthocyanidins associated with the hull fraction of the seed were found to have a minimal or no anti-nutritive effect on nutrient utilization and growth performance (Slominski et al., 2012; Jia et al., 2012).

Dietary fibre content of feed ingredients

The chemical composition of feed ingredients shown in Table 5 represents a typical quality evaluation approach in the author's laboratory. The composition includes all major nutrients and fibre components. The analysis of fibre fractions, as per Manitoba method, accounts for all very important components which individually or collectively influence the nutritive value of feedstuffs. As can be seen, barley, wheat and corn DDGS, SBM and canola meal show higher values for NSP and consequently higher values for TDF. The TDF values for corn and wheat are significantly lower than those for other products due to the much lower content of NSP.

The various types of polysaccharides may be rationalized from the component sugar profile (Table 5). In cereal grains arabinoxylans predominate (Henry, 1987), although significant amounts of β -glucans and cellulose are also present, especially in barley. The relatively high concentration of uronic acid along with glucose residues indicates that pectic-type substances and cellulose are the major cell wall constituents of vegetable proteins (Siddiqui and Wood, 1977; Bacic et al., 1988; Bach Knudsen, 1997). It would appear that the bulk of arabinose and galactose not associated with pectic substances derives from arabinan and arabinogalactan, while xylose indicates the presence of xylan and xyloglucan. Although the structural features of polysaccharides containing mannose units are not fully elucidated, it seems probable that these polysaccharides are structurally similar to galactomannans which have been shown to be widespread in the seeds of leguminous plants (Aspinal, 1982).

In comparison to SBM and canola meal, both yellow peas and low-tannin faba beans show lower contents of NDF and TDF suggesting their good nutritive value. In recent years, low-tannin faba beans have been successfully used in diets for pigs (Zijlstra et al., 2008) and broiler chickens (Vilarinho et al., 2009). It has been demonstrated that chickens tolerate 20% of conventional faba bean seeds in their diets (Nalle et al., 2010), and up to 30% of low-tannin faba bean having no adverse effect on growth performance, as compared to the control SBM-based diet (Usayran et al., 2014).

Table 5. Chemical composition of feed ingredients with special reference to dietary fibre components (10% moisture basis)

Ingredient	Wheat	Barley	Corn	Wheat DDGS	Corn DDGS	SBM	Canola meal	Expelle d CM	Peas	Faba beans
Crude protein	12.8	10.9	7.7	36.7	27.5	46.6	36.8	33.9	20.1	25.4
Ether extract	1.2	1.8	3.7	4.0	9.7	1.4	3.9	11.0	1.3	1.1
Carbohydrates										
Simple sugars	0.2	0.2	0.2	0.9	1.9	1.0	0.3	0.1	0.4	0.2
Sucrose	1.0	1.3	1.2	0.0	0.0	6.2	5.6	5.0	1.6	1.2
Oligosaccharides	1.3	0.8	0.5	0.0	0.0	5.6	2.3	2.7	3.2	1.4
Starch	57.9	53.7	65.4	3.0	5.4	1.6	0.9	0.6	41.8	38.7
Fibre fractions										
Acid detergent fibre	3.2	5.8	2.8	10.3	13.4	7.5	16.1	14.8	6.8	8.4
Neutral detergent fibre	9.2	15.9	7.8	24.0	29.4	12.2	27.0	24.8	9.5	11.3
Total dietary fibre	12.3	22.7	10.2	30.2	32.0	19.3	35.0	32.1	17.0	16.8
Non-starch polysaccharides	9.1	17.9	7.5	19.6	20.1	14.7	20.3	17.7	12.5	13.6
Rhamnose	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.2	0.1	0.1
Arabinose	2.1	2.1	1.5	4.4	4.3	2.4	4.1	3.7	2.6	1.8
Xylose	3.4	4.8	1.9	7.1	6.7	1.0	1.6	1.5	0.9	1.1
Mannose	0.1	0.3	0.0	1.2	1.0	0.6	0.4	0.3	0.1	0.1
Galactose	0.3	0.2	0.4	0.6	1.0	4.4	1.4	1.3	0.5	0.4
Glucose	3.0	11.1	3.2	5.6	5.9	3.2	6.4	5.3	5.8	7.2
Uronic acids	0.4	0.4	0.6	0.7	1.3	3.0	6.3	5.4	2.6	3.0
Lignin and polyphenols	2.1	3.8	2.1	7.5	7.3	3.0	9.9	9.1	3.2	2.6
Glycoproteins (NDICP ¹)	1.1	1.0	0.6	3.1	4.7	1.5	4.8	4.4	1.3	0.6
Ash	2.1	2.3	1.4	4.5	3.7	6.3	6.7	6.8	2.5	2.7
Total phosphorus (P)	0.36	0.34	0.28	0.94	0.75	0.65	1.02	0.92	0.35	0.43
Phytate P	0.28	0.24	0.21	0.18	0.20	0.35	0.61	0.61	0.22	0.15
Non-phytate P	0.08	0.10	0.07	0.76	0.56	0.30	0.41	0.31	0.13	0.28

¹Neutral detergent insoluble crude protein

'Resistant starch' of pulses

It is well known that starch is almost completely digested in the small intestine by a series of α - and β -amylases. Studies with monogastric animals have shown that less than 5% of dietary starch escapes digestion in the small intestine ((Moran, 1982; Meng et al., 2004). Feed manufacturing procedures employing heat and moisture (i.e., drying, extrusion, pelleting) improve starch digestion.

In some studies, however, ileal and total tract digestibilities of starch in broiler chickens fed the corn-peas diet averaged only 87 and 93%, respectively, and were significantly lower than those of the corn-SBM diet (Meng and Slominski, 2015). Under microscopic examination, Wursch et al. (1986) demonstrated that pea starch, together with associated protein, is located in

parenchyma cells with thick walls, which may limit starch digestibility due to starch encapsulating effect of the cell walls. When compared with cereal grain starches, pea starch has a higher amylase-to-amylopectin ratio. According to Daveby et al. (1998), amylose is relatively less digestible by nonruminants than amylopectin. Also, in pea starch a C pattern granule predominates, which is more resistant to pancreatic amylase than the A pattern typical for cereal grains (Canibe and Bach Knudsen, 1997; Daveby et al., 1998). Therefore, when compared with the other diets, the relatively lower starch digestibility of the pea diet measured at the ileal or fecal level may reflect the resistant nature of pea starch to digestion by monogastric animals. As demonstrated by Bach Knudsen et al. (2013), over a wide range of concentrations of dietary fibre in natural feedstuffs, the level of dietary fibre had no impact on the digestibility of starch in the small intestine, except for dietary fibre from legumes where the cell walls may encapsulate potentially available starch within the cells as is the case for peas and faba beans. In this context, the relatively low degree of digestibility of starch in pulses, including peas and beans, has been indicated to contribute to gastrointestinal function and health, and its positive impact on the postprandial glucose response and low glycemic index in humans has been demonstrated (Dahl et al., 2012).

Low starch digestibility may also be the case for lentils (41% starch, 21.9% TDF) and chickpea. In the latter case, nutrient digestibility could be different due to significantly different fibre contents in chickpea varieties. As an example, chickpea var. Desi and var. Kabuli would contain the same amounts of protein (~20%) but would significantly differ in the content of starch (32% Desi; 45% Kabuli) and NDF (21% Desi and 10% Kabuli).

Neutral detergent insoluble crude protein (NDICP)

The polymeric aggregates resulting from Maillard reaction, often referred to as neutral detergent insoluble crude protein (NDICP), appear to be relatively high in processed feedstuffs such as wheat and corn DDGS, and canola meal (Table 5). As they often resemble lignin, they are also included in the lignin and polyphenol content, the fraction which again is the highest in these processed feed ingredients. This is because initially Maillard products contain one amino acid and one sugar molecule. Therefore, it is reasonable to assume that Maillard products contain about 8% N rather than 16% N like in protein and 6.25 coefficient is most likely too small to convert N into total mass of the advanced glycation end product (Martens, 2015).

As documented in Table 5 for 198 samples of canola meal collected from 11 Canadian crushing plants over 6 consecutive years (3 samples per plant, per year), differences between processing facilities in the contents of NDF and TDF were observed with NDICP and lignin with associated polyphenols showing the highest variation relative to the mean values. There were positive correlations between NDF and total dietary fiber ($R^2 = 0.97$), and dietary fiber and NDICP contents ($R^2 = 0.79$). Also, there were significant negative correlations between total dietary fiber and lysine ($R^2 = 0.64$), lysine and NDF ($R^2 = 0.53$), and lysine and NDICP ($R^2 = 0.48$).

This is a clear indication that the variation in the NDF, lignin and NDICP contents is a consequence of excessive heating and the formation of Maillard reaction products and indicative of protein damage leading to the increase in the analysed NDF, lignin and NDICP contents and

reduced protein digestibility. As indicated earlier, the analysed concentration of lignin with associated polyphenols may increase because some melanoidins, which are polymers originating from Maillard reaction, may be analysed as lignin. Consequently, heat treatment of feed ingredients is expected to increase the analysed values of NDF and total dietary fiber (Almeida et al., 2014). This indicates that the variation in the NDF content between processing facilities would mainly be due to differences in heat treatment applied during the desolventization/toasting and drying of the meal (Adebole et al., 2016). As determined in the in vivo studies standardized ileal digestible contents of lysine, threonine, and arginine ranged from 1.57 to 2.01, 0.90 to 1.31 and from 1.97 to 2.31 % DM, respectively, for poultry (Adebole et al., 2016) and from 1.61 to 1.95, 0.94 to 1.27, and from 2.00 to 2.37 % DM, respectively, for swine (Adebole et al., 2017).

Table 6. Effect of processing plant on dietary fibre content of canola meal (% DM)

Component	Mean (n=198)	Range
		Min. – Max.
Neutral detergent fibre	30.0	23.3 – 36.5
Total dietary fibre	38.9	30.1 – 44.3
Non-starch polysaccharides	24.0	19.1 – 25.4
Glycoprotein (NDICP)	5.4	2.4 – 9.6
Lignin and polyphenols	11.0	7.7 – 14.1

Wheat by-products

Wheat milling by-products are usually marketed as wheat mill run or wheat middlings containing a composite of various fractions of the wheat kernel (i.e., wheat shorts, red dog, wheat bran). The difference between wheat mill run and wheat middlings is in the lower proportion of the wheat bran present in the latter product. Bakery products are a by-product of the bakery and confectionary industry and consist of the mixture of bread, cookies, cake, crackers, candy, inedible flours, unsalable nuts and dried doughs that have been mechanically separated from non-edible material (Canada Feeds Regulations 1983). Bakery products are often preferred ingredients in broiler chicken and starter pig diets presumably because of their relatively high content of available energy deriving from starch, sugars and fat. Wheat screenings is a by-product of the cleaning and grading of wheat and is composed of thin, broken and shrunken wheat kernels, weed seeds and other contaminants including straw, chaff, and dust. As defined by the Canada Feeds Regulations (1983), No.1 wheat screenings shall contain no less than 35% grain, not more than 8% small weed seeds (i.e., wild and domestic mustard, flax, canola seed), and not more than 8% wild oats.

Wheat by-products can be extremely variable in composition and consequently are recommended for use in diets of young poultry and pigs at moderate inclusion rates of up to 10% (Leeson and Summers 1991). Wheat by-products such as wheat bran and wheat middlings are higher in CP and AA content when compared to wheat. The difference in wheat milling technologies contributes to a great variability in the content of protein-rich aleurone layer, germ, and starchy endosperm and thus ME and other nutrients content (Cromwell et al., 2000). The

accumulation of fibre may have a negative impact on nutrient digestibility in pigs (Rosenfelder et al., 2013), most likely due to nutrient encapsulating effect of cell walls.

In general, wheat middlings contain high amounts of NDF, NSP, and TDF (Table 7). As reviewed by Rosenfelder et al. (2013), the higher fibre content in these wheat by-products is associated with lower SID for all AA in pigs. Therefore, the fiber fraction should be an important consideration for predicting the ME of feedstuffs (Noblet and Perez, 1993). Dale (1996) reported that NDF was the only component of proximate composition significantly correlated with true metabolizable energy (TME) of wheat by products for broilers. Furthermore, Wan et al. (2009) developed a prediction equation based on NDF being the best single predictor of TME for ducks ($TME = -0.1564NDF + 17.4696$, $R^2 = 0.94$, $P = 0.0003$).

Table 7. Chemical composition of wheat by-products with special reference to protein, fat, starch, dietary fibre and available energy and amino acids contents (10% moisture basis)

Item	Wheat mill run ¹		Bakery by-products		Wheat screenings	
	Mean	Range	Mean	Range	Mean	Range
Crude protein						
<i>Cromwell et al., 2000</i>	16.3	14.6-17.8	-	-	-	-
<i>Slominski et al., 2004</i>	16.5	14.1-18.2	11.9	10.3-14.1	15.1	13.6-17.7
<i>Wan et al., 2009</i>	18.4	16.5-20.1	-	-	-	-
<i>Rosenfelder et al., 2013</i>	-	14.2-22.1	-	-	-	-
<i>Adedokun et al., 2015</i>	17.0	-	11.6	10.6-12.5	-	-
Ether extract						
<i>Slominski et al., 2004</i>	4.3	3.2-5.8	8.0	4.2-10.2	6.6	2.9-20.5
<i>Wan et al., 2009</i>	3.9	3.2-4.7	-	-	-	-
<i>Rosenfelder et al., 2013</i>	-	2.7-6.5	-	-	-	-
<i>Adedokun et al., 2015</i>	-	-	7.1	3.9-8.4	-	-
Starch						
<i>Slominski et al., 2004</i>	26.4	16.2-30.4	37.8	24.7-46.5	45.7	27.9-54.4
<i>Rosenfelder et al., 2013</i>	-	13.9-71.6	-	-	-	-
Neutral detergent fibre						
<i>Cromwell et al., 2000</i>	36.9	29.9-43.9	-	-	-	-
<i>Wan et al., 2009</i>	34.8	16.6-52.9	-	-	-	-
<i>Rosenfelder et al., 2013</i>	-	6.7-48.2	-	-	-	-
<i>Adedokun et al., 2015</i>	39.9	-	13.8	6.6-19.5	-	-
Total dietary fibre						
<i>Slominski et al., 2004</i>	38.7	33.8-46.2	13.4	6.9-32.7	19.4	17.6-20.6
Non-starch polysaccharides						
<i>Slominski et al., 2004</i>	22.4	20.1-28.2	8.7	3.3-17.0	10.0	7.2-11.8
<i>Rosenfelder et al., 2013</i>	-	18.3-41.6	-	-	-	-
TME _n , kcal/kg (poultry)						
<i>Slominski et al., 2004</i>	2,346	-	3,425	3,091-3,757	3,150	3,076-3,263
<i>Wan et al., 2009</i>	2,871	2,109-3,597	-	-	-	-
ME, kcal/kg (swine)						
<i>Rosenfelder et al., 2013</i>	-	2,412-3,798	-	-	-	-
AA digestibility, %						

SID lysine						
<i>Adedokun et al., 2015</i>	69	-	72	66-79	-	-
<i>Rosenfelder et al., 2013</i>	-	68-91	-	-	-	-
SID threonine						
<i>Adedokun et al., 2015</i>	59	-	74	68-81	-	-
<i>Rosenfelder et al., 2013</i>	-	68-90	-	-	-	-
SID methionine						
<i>Adedokun et al., 2015</i>	68.8	-	83	80-86	-	-
<i>Rosenfelder et al., 2013</i>	-	72-80	-	-	-	-

[†]Includes wheat middlings, wheat bran, wheat shorts, red dog.

High NSP and total fibre contents of mill run would appear to make this product less attractive for poultry feeding, although high protein content along with appropriate enzyme supplement (to target the fibre components) could make this by-product a valuable ingredient in monogastric animal rations. Bakery by-products, on the other hand, could serve as a worthwhile energy supplement in poultry and swine diets, although high variability in sugars, starch, and fat content would necessitate rigorous quality control programs to ensure optimal growth performance by monogastric animals. High levels of oilseeds in wheat screening may necessitate more effective grinding procedures as the small size of the cells within the cotyledon fraction of the seed may limit oil (energy) and protein utilization. Overall, grinding, pelleting and enzyme supplementation may further increase the feeding value of wheat by-products.

As an example, the effect of enzyme supplementation was evaluated in the author's laboratory with broiler chickens fed a positive control (PC) (wheat/soybean meal), negative control (NC) (wheat/wheat by-products/barley/soybean/ canola meal) and NC + multi-enzyme diets (Boros et al., 2004). In comparison to PC, the NC diet contained 5% less metabolizable energy, 8–10% less crude protein, amino acids and calcium and 25% less available phosphorus. Body weight gain and feed conversion ratio averaged 2.14, 2.10, 2.17 kg/bird and 1.68, 1.76, 1.66 for PC, NC and NC + Enzyme, respectively. A significant ($P<0.05$) improvement in broiler chicken performance with enzyme supplementation was further substantiated by the same magnitude of difference in dry matter (71.2, 68.7, 75.1%), starch (92.5, 90.3, 95.6%), and NSP (4.8, 15.0, 36.1%) digestibilities, and diet AME_n content (2,955, 2,826, 3,067 kcal/kg). It is evident from this study that the use of wheat by-products in concert with an effective dietary enzyme supplement could allow for optimum growth performance.

Dietary fibre and efficiency of animal production

Several effects of dietary fibre in relation to efficiency of animal production have been well documented. These include:

- 1) Low nutrient density of high-fiber feedstuffs,
- 2) Antinutritive effects of water-soluble NSP (i.e., β -glucan, arabinoxylan),
- 3) Nutrient encapsulating effect of cell walls,
- 4) Impaired amino acid availability (i.e., Maillard products),
- 5) Benefit from short chain fatty acids (SCFA) production, and
- 6) Prebiotic effects of NSP and NSP hydrolysis products.

Non-starch polysaccharides of feed ingredients can pose a viscosity problem (more so in poultry than swine), encapsulate nutrients, and thus affect overall nutrient absorption. The water-soluble and viscous β -glucans and arabinoxylans present in barley, rye, and wheat interfere with the mixing of digestive enzymes and nutrients and impede digesta movement and transport of hydrolysis products to the intestinal mucosa. As a result, these effects may cause a decrease in animal performance. In addition, management problems related to “sticky droppings” have been indicated to be directly associated with the high water-holding capacity of β -glucans and arabinoxylans. To counteract such antinutritional effects, many commercial preparations of β -glucanase and xylanase have been developed over the past 30 years.

In addition to viscosity reduction, the use of effective combinations of NSP-degrading enzymes could reduce the nutrient encapsulating effect of cell walls which, in turn, would contribute to increased protein, starch, and fat utilization as demonstrated by studies on wheat (Tervila-Wilo et al., 1996, Bedford and Autio, 1996), full-fat canola (Meng et al., 2006), full-fat flaxseed (Slominski et al., 2006), or expelled full-fat soybeans (Ayoade, 2012).

Using flaxseed as an example, the elimination of the nutrient (fat) encapsulating effect of cell walls by enzyme supplementation has been very well documented. Full-fat flaxseed contains approximately 40% oil and is an attractive feed ingredient in Canadian poultry diets because of its high content of ω -3 unsaturated fatty acids (48 to 58% of the oil), which can be deposited in egg or meat products and can have a positive effect on human health. However, reduced energy use and depressed growth and feed efficiency have been observed when incorporating ground flaxseed into poultry diets. Lee et al. (1991) found that the use of equal portions of flax meal plus flax oil in place of ground flaxseed, significantly improved body weight gain, feed efficiency, and dietary AME_n. The less-than-optimum energy use from full-fat flaxseed was a result of limited oil availability, because in conventionally ground flaxseed, a substantial amount of oil would be encapsulated by the cell wall NSP.

As illustrated in Table 8, an increase ($P < 0.05$) in TME_n content from 2,717 to 3,751 kcal/kg was observed for the flaxseed supplemented with a multi-carbohydrase preparation specifically developed to target NSP of this ingredient. A similar pattern of increase in fat and NSP digestibilities was noted. This was further substantiated in a trial in which commercial samples of flaxseed were subjected to energy evaluation.

Table 8. Effect of multi-carbohydrase enzyme addition on digestibility of fat, non-starch polysaccharides (NSP) and available energy (TME_n) content of laboratory hammer-milled full fat flaxseed when fed to adult roosters

Enzyme	Digestibility, %		TME _n kcal/kg
	Fat	NSP	
None (control)	59.4	12.9	2,717
Multi-carbohydrase	74.2	35.3	3,751

Table 9. Effect of multi-carbohydase enzyme supplementation on available energy (TME_n) content of commercial samples¹ of full-fat flaxseed products

Sample	TME _n , kcal/kg
Flaxseed (hammer milled)	2,205
Flaxseed (hammer milled) + Multi-carbohydase	3,300
Flaxseed “mix” (60% flaxseed; extruded)	3,237
Flaxseed “mix” (60% flaxseed; extruded) + Multi-carbohydase	3,610

¹Samples were obtained from feed manufacturers in Manitoba and Quebec and were reported to be used in laying hen rations for ω -3 egg production.

As illustrated in Table 9, neither conventional hammer milling nor extrusion of flaxseed was as effective in increasing the available energy content as dietary enzyme supplementation. This result is indicative of incomplete rupture of the seeds during the conventional hammer milling process. It would appear evident from this study that following conventional hammer-mill grinding or extrusion, some portion of the oil is still encapsulated by the cell wall structure, and enzyme supplementation could further facilitate the seed ‘digestion’, which, in turn, would result in optimum energy use from full-fat flaxseed.

Dietary NSP also interact with gut microflora which could be considered beneficial from the point of view of energy utilization and gut health. The extent of microbial breakdown of NSP is influenced by the NSP composition, solubility in water and the degree of cell wall lignification. The polysaccharide fractions are degraded to a much greater extent in the large intestine of pigs (~54%) than that of poultry (~6%). The relatively low digestibility of NSP in poultry indicates that the ceca do not contribute significantly to the digestion of dietary fibre. The main end products of microbial fermentation are short chain fatty acids (SCFA) which provide the host with energy. In this regards, swine produce SCFA that provide up to 8% of their energy requirement. In poultry, the contribution of energy derived from SCFA has been reported as very small relative to the total need (i.e., 3%). Therefore, higher dietary fibre at the expense of starch and sugars has negative implications for the net energy value of the feed.

Prebiotic effects of NSP and NSP-hydrolysis products

Water-soluble components of dietary fibre may have an important influence on gut health, and similarly to prebiotics, may facilitate proliferation of bacteria beneficial for gut health such as *Bifidobacterium* and *Lactobaccillus*, thereby decreasing the abundance of pathogens such as *Clostridium*, *Salmonella*, *E. coli* and *Campylobacter*. In this context, water-soluble NSP may indirectly prohibit the growth of certain pathogenic species by increasing acidity through an increase in lactic acid in the lower gut. Concomitantly, non-substrate utilizers, in a highly competitive ecosystem, will be suppressed and can virtually disappear. In addition, certain NSP or NSP hydrolysis products may attract microbes away from the intestinal binding sites by a means of competitive exclusion, thereby reducing colonization and disease and allowing the mucosa to perform its function of secretion, digestion and nutrient absorption.

These water-soluble fibre components would increase both ileal and post-ileal microbial fermentation with the production of considerable amounts of SCFA. Among SCFA, butyrate has

been implicated as having a role in animal health. As reviewed by Montagne et al. (2003), butyrate stimulates the development and growth of the small and large intestine, by stimulating epithelial cell proliferation. In the large intestine, SCFA stimulate the resorption of water and sodium, thus limiting the risk of diarrhoea. Butyric acid has also been implicated in the control of enteric pathogens such as *Salmonella* and *Clostridium perfringens* and has also been reported to have anti-inflammatory properties (Van Immerseel et al., 2005).

Somewhat similar mechanism of action would be expected from the production of NSP hydrolysis products in the presence of dietary enzyme supplements. In the process of depolymerizing various NSP in the diet, carbohydrase enzymes may produce a variety of low-molecular weight polysaccharides, oligosaccharides and simple sugars which may acquire the potential to become prebiotics and can exert health benefits by improving the intestinal environment.

In the most recent study from this laboratory (Kozłowski et al., 2018), it was demonstrated that the addition of a multi-carbohydrase preparation was effective in promoting growth and feed utilization of turkeys fed antibiotic-free diets. Enzyme supplementation increased the concentration of SCFA in cecal digesta with an increase in acetic and butyric acids, the latter one known to be beneficial in controlling enteric pathogens and in improving gut health. In this context, an interesting concept has recently been proposed to modulate endogenous butyrate production by dietary means (Onrust et al., 2015), as following fermentation different sources of fiber would yield different levels of butyrate.

Table 10. Growth performance and short chain fatty acid (SCFA) content in cecal digesta of growing turkeys (1 to 8 weeks) fed diets without or with multi-carbohydrase supplementation

Diet	BWG (kg/bird)	FCR	SCFA (μmol/g digesta)		
			acetic	butyric	total
Control	3.85	1.71 ^a	94.2 ^b	32.1 ^b	136.0 ^b
Enzyme	3.92	1.66 ^b	110.0 ^a	36.3 ^a	158.0 ^a

^{ab} $P < 0.05$

Concluding Remarks

Facts about dietary fibre in relation to animal production would reflect the phrase “The Good, the Bad and the Ugly” with insoluble NSP decreasing transit time, enhancing water holding capacity and assisting faecal bulking, whereas soluble NSP having positive effects on microbiota composition, SCFA production, control of enteric bacterial pathogens (prebiotic effect), and ‘gut health’ when fed along with enzyme supplements. Undoubtedly lignin is “the bad” fiber component as it would contribute to nutrient encapsulating effect of cell walls, their low biodegradability, and low nutrient density of high-fiber feedstuffs. Products of final stages of Maillard reaction would definitely belong to “the ugly” category due to losses of available lysine, threonine, arginine, and inhibiting effects on trypsin and intestinal brush border enzymes and proliferation of beneficial bacteria.

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Effects of Pre-Weaning Iron Supplementation on Post-Weaning Disease Challenge, Growth Performance and Antibody Response in Pigs

Effets de la supplémentation en fer avant le sevrage sur la santé, la performance de croissance et la réponse immunitaire après le sevrage chez les porcs

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Abstract

In commercial pig husbandry it is a common practice to administer iron dextran (200 mg per piglet) within the first few days post-farrowing in order to prevent anemia. However, in modern day production, fast growing pigs are often iron deficient at 3 weeks of age. Within the first week in the nursery, there are many stressors and disease-challenges. It is the objective of this research to determine if pigs with iron deficiency at weaning are more susceptible to enteric disease and if they have a reduced antibody response to *Mycoplasma hyopneumoniae* vaccine and poorer growth rate compared to pigs with adequate iron stores. At three days of age, piglets (n=254) from 22 litters were assigned to a low, medium or high iron dose. A subset of pig DNA was extracted from 164 blood samples and subjected to RFLP-PCR to determine the pig susceptibility to F4 ETEC. A blood sample was taken at weaning to determine the hemoglobin concentration. Susceptible pigs (n=72) were transported to a level 2 biosafety facility and in each of 2 trials, 6 pigs (2 pigs from each iron treatment) were placed in each of 6 rooms. On Day 2 post-weaning, in each trial 5 rooms were challenged with 2 mL of 10^9 CFU of F4 enterotoxigenic *E. coli*. After 2 days post-challenge pigs were euthanized, and intestinal tissue samples were collected for histological examination. Another subset of these pigs (n=145) were categorized based on their hemoglobin levels at weaning (anemic, iron deficient and normal) and followed throughout the entire nursery period. Weights and blood samples were taken at 3, 6 and 9 weeks of age. It was concluded based on clinical signs of disease, post-mortem sampling, antibody response and growth rate, there was no difference among the three iron categories of pigs at weaning with respect to their performance in the nursery ($P>0.05$).

Résumé

Dans les exploitations porcines commerciales, l'administration de fer-dextran (200 mg par porcelet) dans les premiers jours qui suivent la naissance est couramment pratiquée pour prévenir l'anémie. Toutefois, dans les conditions de production moderne, les porcs à croissance rapide présentent souvent une carence en fer à l'âge de trois semaines. De nombreux facteurs de stress et agents pathogènes apparaissent durant la première semaine en pouponnière. L'objectif de cette étude est de déterminer si les porcs atteints d'une carence en fer au sevrage sont plus sensibles aux affections entériques, s'ils répondent moins au vaccin contre *Mycoplasma hyopneumoniae* et

s'ils croissent moins rapidement que les porcs dont les réserves en fer sont suffisantes. À l'âge de trois jours, les porcelets (n=254) de 22 portées ont reçu une dose de fer faible, intermédiaire ou élevée. L'ADN de porc a été extrait d'un sous-ensemble de 164 échantillons de sang et a fait l'objet d'une analyse RFPL-PCR afin de déterminer la sensibilité des porcs à ETEC F4. Un prélèvement sanguin a été effectué au sevrage pour mesurer la concentration d'hémoglobine. Les porcs sensibles (n=72) ont été transportés dans une enceinte de biosécurité niveau 2 et, dans chacun des deux essais, six porcs (deux porcs de chaque traitement de fer) ont été placés dans six salles. Au jour 2 après le sevrage, dans chaque essai, cinq salles ont été exposées à 2 mL de 10^9 CFU de *E. coli* entérotoxigène F4. Deux jours après l'exposition, les porcs ont été euthanasiés et des échantillons de tissus intestinaux ont été recueillis aux fins d'examen histologique. Un autre sous-groupe de ces porcs (n=145) a été formé sur la base de leur concentration en hémoglobine au sevrage (anémique, carencé et normal) et ces animaux ont fait l'objet d'un suivi durant toute la période passée en pouponnière. À l'âge de trois, six et neuf semaines, les animaux ont été pesés et des échantillons de sang ont été prélevés. L'analyse des signes cliniques de maladie, des échantillons de tissus post-mortem, des réponses immunitaires et des taux de croissance permet de conclure à l'absence de différence entre les trois groupes de traitement en fer au sevrage en ce qui a trait à la performance des porcs dans la pouponnière ($P>0,05$).

Introduction

Neonatal piglets require iron supplementation within the first week of life and although intramuscular administration of 200 mg of iron dextran is routine practice on almost all modern swine farms, iron deficiency resulting in anemia can occur (Murphy et al., 1997; Bruininx et al., 2000; Svoboda, M., Drabek, 2005). Weaning is a stressful time and it is not uncommon for newly weaned pigs to face disease challenges (Oosek et al., 1983; Madec et al., 2000). Iron deficiency might interfere with the pig's immune system and cause pigs to be at risk during the first few weeks after weaning (Schrama et al., 1997).

Objectives:

1. To determine if pigs with iron deficiency at weaning are more susceptible to develop post-weaning diarrhea after an experimental *E. coli* challenge compared to pigs with adequate iron levels.
2. To determine whether iron status (based on hemoglobin levels at weaning) has an effect on antibody response to *Mycoplasma hyopneumoniae* vaccine and on growth rate in nursery pigs.

Materials and Methods

Two trials were conducted. For each trial all piglets from 11 litters at the Arkell Research Station were ear tagged at 2 days of age. At 3 days of age, one-third of the litter were given 100 mg of iron dextran (Uniferon[®]200, Pharmacosmos Inc.) by intramuscular injection, and the remaining two-thirds of the litter were given 200 mg of iron dextran. One-third of the pigs that received 200

mg of iron on Day 3 were injected with a second dose of 200 mg of iron on Day 14. Piglets were not provided with creep feed for the purpose of this study.

Enterotoxigenic *E. coli* (ETEC) challenge study:

At 9 days of age, pig DNA was extracted from blood samples collected from a subset of pigs and subjected to RFLP-PCR to determine the presence of receptor for F4 ETEC. At weaning, 12 susceptible pigs from each treatment (total of 36 pigs) were weighed and a whole blood sample was taken from each pig and hemoglobin determination performed using the ADVIA 2120/2120i Hematology System (Siemens Healthcare Diagnostics, Deerfield, IL, USA) at the Animal Health Laboratory (AHL), University of Guelph. Piglets were then transported to the Ontario Veterinary College (OVC) Isolation Facility and housed in 6 rooms (2 pigs from each iron treatment in each room). One day after arrival, pigs in 5 rooms were challenged by oral gavage with 2 mL of 10^9 CFU of *E. coli* O149:K91:F4. Prior to and post-challenge the clinical observations (diarrhea score, depression, dehydration, temperature, and appetite) were recorded and fecal swabs were taken and cultured for ETEC. Pigs were euthanized two days after challenge and tissue samples were taken from the jejunum, ileum and colon for histological examination; colon samples were also taken and cultured for ETEC.

Growth Performance and Antibody Response Study:

Over two separate trials a subset of piglets (n=145) at weaning were moved to the nursery and followed for 6 weeks. All of these pigs were vaccinated against *Mycoplasma hyopneumoniae* at weaning and revaccinated 3 weeks later. Pigs were weighed, and blood samples were taken at weaning, 6 weeks of age (prior to receiving the second *M. hyopneumoniae* vaccine) and 9 weeks of age. Blood samples were submitted to AHL to determine hemoglobin levels and serum samples were tested for presence of antibody to *M. hyopneumoniae* using an enzyme-linked immunosorbent assay (IDEXX, Markham, ON).

Results

ETEC Challenge Study:

At weaning 72 piglets in two trials were categorized based on their hemoglobin levels as anemic (<90 hgb/L; n=17), iron deficient (90 to 110 hgb/L; n=18), and normal iron levels (>110 hgb/L; n=37). Over the course of three collection periods post-challenge, 53% of anemic pigs, 57% of iron deficient pigs and 55% of the pigs with adequate iron levels had diarrhea. No significant difference in overall response to ETEC challenge was observed among pigs with three different pre-weaning iron supplementation doses or with different levels of hgb determined at weaning ($P>0.05$).

Growth Performance and Antibody Response Study:

At weaning, 145 piglets were categorized based on their hemoglobin (hgb) levels into three groups including anemic (<90hgb/L; n=33), iron deficient (90-110 hgb/L; n=36), and normal

(>110 hgb/L; n=76). Among the anemic and low iron groups, hgb levels increased during the nursery phase and prior to exit from the nursery. The majority of pigs were classified as having adequate hgb levels (n=134) and the remaining pigs were classified as being iron deficient (n=11). In trial 2, pigs that had a normal iron status upon nursery exit had improved overall average daily gain in the nursery ($P<0.05$). The iron status at weaning did not affect the pig's response to vaccination with *M. hyopneumoniae* vaccine ($P>0.05$).

Conclusion

The iron status of weaned pigs at three weeks of age appears to have little effect on the health status of pigs during the nursery phase based on antibody response to vaccination and ability to deal with an enteric pathogen challenge. Iron status at the beginning of the nursery phase does not appear to influence subsequent growth unless hemoglobin levels remain low throughout the nursery phase. However, the pigs in this study were sourced from a research station where a low level of zinc is included in their starter rations. Further studies are needed to investigate the impact of iron on health and growth performance under commercial farming conditions where a higher zinc in the feed is likely used and multiple pathogens may be present.

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Dietary Starch and Fiber as Prebiotics in Swine Diets

Utilisation de l'amidon et des fibres alimentaires comme prébiotiques dans l'alimentation des porcs

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Abstract

Dietary antibiotics will be used less as growth promotant; thus, alternatives are investigated. Some efforts are focused on using feed additives as alternatives, but macronutrients in feedstuffs may also be part of a toolkit to manage gut health in pigs. Dietary carbohydrates include starch, fiber (non-starch polysaccharides) and oligosaccharides. Antibiotics are hypothesized to control gut health via manipulations of intestinal microbial profiles or reduced intestinal inflammation. Starch is mostly digested and absorbed as glucose; however, resistant starch is not digested but fermented. Resistant starch acts as fiber but is unique, because it 1) specifically increases digesta bifidobacteria that have been associated with improved gut health and 2) is completely fermented within the gut. Sources of fiber differ in 2 key characteristics: viscosity and fermentability. Increased viscosity has been associated with increased gut content of virulence factors that are linked with diarrhea. Increased kinetics of fiber fermentation is associated with changes in microbial profiles and increased metabolite production. Oligosaccharides may be rapidly fermented and thereby influence intestinal microbial profiles and metabolite production. Specific exopolysaccharides from *Lactobacillus reuteri* may serve as scavenger molecules for pathogenic bacteria, e.g., enterotoxigenic *E. coli* (ETEC). The ETEC binding to exopolysaccharides may avoid diarrhea initiation by ETEC. Recently, microbial composition was hypothesized to be less important and the focus should be on their combined output of metabolites. Raw materials and prebiotic feed additives both influence kinetics of fermentation and have prebiotic activity. Their kinetics of fermentation should be quantified so that it can be included in feed formulation. In conclusion, dietary carbohydrates via their prebiotics activity are part of the solution to remove antibiotics as growth promotant from swine diets.

Résumé

Les antibiotiques seront moins utilisés comme facteurs de croissance dans l'alimentation; c'est pourquoi des solutions de rechange sont étudiées. Certains travaux portent sur l'utilisation d'additifs alimentaires comme solutions de remplacement, mais les macronutriments des ingrédients alimentaires pourraient aussi faire partie du coffre à outils pour la gestion de la santé gastro-intestinale chez les porcs. Les hydrates de carbone de la ration incluent l'amidon, les fibres (polysaccharides non amylacés) et les oligosaccharides. On suppose que les antibiotiques interviennent sur la santé gastro-intestinale en modifiant les profils microbiens intestinaux ou en réduisant l'inflammation intestinale. L'amidon est surtout digéré et absorbé sous forme de glucose; toutefois, l'amidon résistant à la dégradation intestinale n'est pas digéré, mais plutôt fermenté. L'amidon résistant agit comme la fibre, mais est unique 1) parce qu'il favorise spécifiquement les bifidobactéries du digesta qui ont été associées à l'amélioration de la santé gastro-intestinale et 2) parce qu'il est complètement fermenté dans les intestins. Deux caractéristiques clés distinguent les sources de fibres : la viscosité et la fermentescibilité. Une augmentation de la viscosité a été associée à une augmentation de la teneur du contenu intestinal en facteurs de virulence liés à la diarrhée. L'accélération de la cinétique de la fermentation des fibres est associée à des changements de profils microbiens et à une augmentation de la production de métabolites. Les oligosaccharides peuvent être fermentés rapidement et, par conséquent, peuvent influencer les profils microbiens intestinaux ainsi que la production de métabolites. Certains exopolysaccharides de *Lactobacillus reuteri* pourraient servir de molécules piégeuses de bactéries pathogènes, p. ex., *E. coli* entérotoxigène (ECET). Cet effet des exopolysaccharides pourrait éviter le déclenchement de la diarrhée à ECET. Récemment, on a émis l'hypothèse selon laquelle la composition microbienne serait moins importante et que l'intérêt devrait se porter vers leur production combinée de métabolites. Les matières premières et les additifs alimentaires prébiotiques influencent la cinétique de la fermentation et exercent une activité prébiotique. Leur effet sur la cinétique de la fermentation devrait être quantifié de façon à pouvoir en tenir compte dans la formulation des rations. En conclusion, les hydrates de carbone alimentaires, compte tenu de leur activité prébiotique, font partie de la solution pour éliminer les antibiotiques utilisés comme facteurs de croissance dans l'alimentation des porcs.

Introduction

The gastrointestinal tract in pigs is colonized with a diverse population of bacteria that supports physiological development and immunologic functions (Hill et al., 2002; Chowdhury et al., 2007; Willing and Van Kessel, 2010). However, pigs can have unstable gut microbiomes at various life stages, allowing opportunity for pathogenic bacteria to colonize and cause disease (Pieper et al., 2008, 2009). A combination of digesta passage rate, digestibility, fermentability, and viscosity contributes to nutrient availability and commensal bacteria colonization in the lower gastrointestinal tract (Metzler-Zebeli et al., 2010; Regmi et al., 2011). Starch and fiber may serve as energy source for the intestinal microbiota and thereby modulate its composition. Dietary starch and fiber are important nutrients for pigs but are underappreciated in their complexity.

Starch

Starch ranging from low to high amylose changes from rapidly enzymatically-digestible in the upper gut to fermentable in the lower gut including distal small intestine, respectively, due to changes in physico-chemical characteristics such as viscosity, fermentability, and water-holding capacity (Regmi et al., 2011). Starch can thus be a source of glucose or VFA (also known as short-chain fatty acids) and for the latter is thus similar to fiber. If starch is fermented instead of digested, whole body energy utilization might be affected (Fouhse et al., 2018). In swine nutrition, the paradigm is that non-digestible carbohydrates are a negative dietary factor due to their fermentation and adverse effects on whole body nutrient utilization (Drew et al., 2012). In human nutrition, with health and not ADG as main objective, fermentable or resistant starch is gaining popularity due to suppression of rate of nutrient absorption and stimulation of intestinal health (Bird et al., 2008).

Switching from dietary digestible to resistant starch provides readily available substrates for microbes in the large intestine, and dietary fiber is also used by colonic microbiota as fermentative substrates (Topping and Clifton, 2001). Diets high in amylose or resistant starch favor the production of commensal bacteria such as *Bifidobacterium* and *Lactobacillus* groups in pigs (Brown et al., 1997; Bird et al., 2007). Decreasing in vitro starch digestion rate by increasing amylose content increased available nutrients (starch and protein) in the colon, thereby increasing commensal *Bifidobacterium* spp. in feces (Regmi et al., 2011). Moreover, dietary inclusion of highly viscous carboxymethyl cellulose increased prevalence of *E. coli* virulence factors, indicating the importance of post-ileal nutrient flow on substrate availability in the lower gut for commensal microbial colonization (Metzler-Zebeli et al., 2010). Although certain fiber sources share physico-chemical properties such as viscosity and fermentability, they do not consistently affect small intestinal digestion, large intestinal substrate availability or microbial responses; hence, fiber sources work according to individual fractions rather than shared functional properties (Metzler-Zebeli et al., 2010). Feeding high amylose starch creates an increasing flow of starch into the large intestine and thereby has a bifidogenic effect (Fouhse et al., 2015).

Fiber

Similarly, dietary fiber has a contradictory status in nutritional sciences. In swine nutrition, fibers such as β -glucans and arabinoxylans are regarded as negative dietary factors due to their negative effects on nutrient digestibility and feed intake (de Lange, 2000). In contrast, fiber gains popularity in human nutrition due to their adverse effects on rate of nutrient digestion and, therefore, glycemic index (Jenkins et al., 2002) and stimulatory effects on intestinal health (Kudo, 2004), and is increasingly regarded as a functional food component. Previously, we reviewed fiber digestion, absorption of its metabolites, and the impact of digestive physiology in the pig (Zijlstra et al., 2012). Among labs, our lab also has used in vitro fermentation methods to mimic in vivo digestion of fiber (Jha and Leterme, 2012; Jha and Zijlstra, 2018). Fiber can be important for energy metabolism of the pig. Fiber varies greatly in characteristics such as fermentability and viscosity that are important for its ability to affect physiological functions (Dikeman and Fahey, 2006). In the gut, fiber via its metabolites may directly stimulate

physiological functions via local endocrine responses (Hooda et al., 2010). Obviously, because fiber cannot be digested by the pig itself, it is fermented by the gut microbiota. A combination of digesta passage rate, fermentability, and viscosity that are all influenced by fiber contributes to nutrient availability and commensal bacteria colonization in the lower gastrointestinal tract (Metzler-Zebeli et al., 2010).

Some oligosaccharides and polysaccharides are recognized as prebiotics for human and animal nutrition (Topping et al., 2003; Bach Knudsen et al., 2012). Non-enzymatically digestible oligosaccharides (NDO) are highly fermentable, decrease gastrointestinal pH producing an unfavorable environment to pH-sensitive microbes (Houdijk et al., 2002; Macfarlane et al., 2006; Bach Knudsen et al., 2012). The NDO bring about prececal prebiotic effect in weaner pigs (Houdijk et al., 2002). As pigs age, their microbiome becomes more stable and inclusion of prebiotic feedstuffs, such as resistant starch, NDO, or high amylose starch grain cultivars, have seemingly fewer effects. Supplementing fructan, a NDO, caused only minor changes in digestibility without changes in microbiota (Hedemann and Bach Knudsen, 2010). Interestingly, when resistant starch and NDO were fed together to pigs, additive effects on colon and fecal bifidobacteria numbers were reported (Bird et al., 2009).

Combining soluble and insoluble NSP may cause similar additive effects on ileal bifidobacteria and enterobacteria populations (Owusu-Asiedu et al., 2006). Multiple studies in humans and other animals indicate that dietary resistant starch and NDO have a prebiotic effect across species (Campbell et al., 1997; Djouzi and Andrieux, 1997; Brown et al., 1998; Tuohy et al., 2001). Oat β -glucan, either concentrated or within the cereal matrix, also has a prebiotic effect in pigs (Metzler-Zebeli et al., 2010); dietary β -glucan selectively increases colonic lactobacilli and bifidobacteria (Metzler-Zebeli et al., 2011) promoting butyrate-producing bacteria (Pieper et al., 2008).

Conclusion

Combined, convincing evidence exists that various starch and fiber fractions have a prebiotic effect in pigs. The carbohydrate fractions can be added to diets as purified sources; however, application via the use of unique cereal grain or pulse grain cultivars may create similar benefits (Fouhse et al., 2017a; Fouhse et al., 2017b). However, the singular or additive effects that carbohydrate fractions have on microbial diversity and colonization remain to be elucidated.

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Understanding the Needs of Branch Chain Amino Acids in Broiler Production

Comprendre les besoins en acides aminés à chaîne ramifiée en production de poulet à griller

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Abstract

Valine is the 4th limiting amino acid for broilers fed diets utilizing amino acid contributing ingredients of vegetable origin (Thornton *et al.*, 2006). Ideal amino acid ratio concept is a popular method to express amino acid requirements relative to lysine. Utilizing L-valine in concert with ideal amino acid ratio concept in diet formulation allows nutritionists to meet an optimum digestible valine to lysine ratio while decreasing nitrogen excretion and maintaining cost effective diets. Formulating diets at a digestible valine to lysine ratio below the broiler's need for valine limits their genetic potential in meeting performance objectives. Digestible valine to lysine ratio has been reported to vary among various production periods with ratios ranging from 0.74 to 0.78 (Corzo 2007, 2008; Rostagno *et al.*, 2011). Corzo *et al.* (2011) has reported acceptable performance with the inclusion of L-valine into broiler diets. Price spread among cereal grains, supplemental oil/fat, and protein meals determines the value of L-valine. During times of high fat prices and a wide spread of grain and protein meal prices will led to the inclusion of L-valine supplementation. Isoleucine will become the pressure point in diet formulation with the inclusion of L-valine. Understanding the digestible isoleucine needs of broilers is important to optimize the rate and efficiency of growth when including L-valine in the diet. This presentation will review research on dietary valine and isoleucine of broilers to optimize growth performance and processing yields.

Résumé

La valine est le 4^e acide aminé limitant chez les poulets à griller recevant des rations dont l'apport en acides aminés provient d'ingrédients d'origine végétale (Thornton *et al.*, 2006). Le concept de profil idéal en acides aminés est une méthode couramment utilisée pour exprimer les besoins en acides aminés par rapport à la lysine. Utiliser la L-valine dans le contexte du concept de profil idéal en acides aminés pour la formulation des rations permet aux experts en nutrition animale d'obtenir un rapport valine sur lysine digestible optimal, tout en diminuant la quantité d'azote excrété et en conservant un rapport coût-efficacité intéressant pour les rations. Formuler les rations en fonction d'un rapport valine sur lysine digestible inférieur aux besoins en valine des poulets à griller limite la capacité génétique des oiseaux à atteindre les objectifs de performance. On a montré que le rapport valine sur lysine digestible varie pour diverses périodes de production, l'intervalle se situant entre 0,74 et 0,78 (Corzo 2007, 2008; Rostagno *et al.*, 2011). Corzo *et al.* (2011) ont obtenu une performance acceptable à la suite de l'inclusion de L-valine

dans les rations des poulets griller. L'écart de prix entre les petites céréales, les compléments d'huile ou de gras et les tourteaux protéiques détermine la valeur de la L-valine. Une période de prix élevés pour le gras et d'écart de prix important entre les différents grains et tourteaux de protéines favorisera la supplémentation en L-valine. À la suite de l'inclusion de L-valine, l'isoleucine deviendra le facteur limitant de la formulation des rations. Il est important de comprendre les besoins en isoleucine digestible des poulets à griller pour optimiser le rythme et l'efficacité de la croissance quand on inclut de la L-valine dans la ration. Cet exposé donne un aperçu de la recherche portant sur les besoins en valine et isoleucine des poulets à griller pour optimiser la performance de croissance et le rendement à la transformation.

Introduction

Kidd and Hackenhaar (2005) reported that valine is the fourth limiting amino acid with broilers fed corn-soybean meal and wheat-soybean meal based diets utilizing formulation scenarios with various ingredients. Conversely, the addition of poultry meal or a meat blend product to the diet can result in isoleucine or tryptophan becoming fourth limiting. Dozier et al. (2011) determined that valine and isoleucine become co-limiting for broilers fed diets containing animal protein meals from 2.5% inclusion. With animal protein meal inclusion exceeding 5% of the diet, isoleucine may become the fourth limiting amino acid. Growth rate and feed conversion are more sensitive response criteria than breast meat yield as digestible valine approaches the requirement (Corzo et al., 2007, 2008). Feed-grade production of L-valine has increased due to the demand in swine and recently it has been entering into some broiler formulations due to the popularity of “all-vegetable” diets in antibiotic-free production. L-valine has entered into formulation of broiler diets containing ingredients of vegetable origin up to 0.75 kg per ton during the starter and grower diets.

Valine

Corzo et al. (2007) conducted a series of experiments to determine the fourth limiting amino acid of Ross × Ross 708 male broilers fed diets containing ingredients of vegetable origin and to delineate the digestible valine to lysine ratio from 21 to 42 d of age. In order to confirm that valine is the fourth limiting amino acid of diets consisting of ingredients of vegetable origin, corn-soybean meal diets were formulated to be marginal in dietary isoleucine, valine, arginine, and glycine plus serine. Supplementing valine to the negative control diet (marginal in valine, isoleucine, arginine, and glycine) led to similar body weight gain of broilers fed the positive control diet, but adding isoleucine, arginine, or glycine to the negative control diet had lower ($P \leq 0.05$) body weight gain compared with the birds fed the positive control diet. These results indicated that valine is the fourth limited amino acid for broilers fed diets containing corn and soybean meal. In a subsequent study, corn-peanut meal negative control diet was formulated to be adequate in all amino acids with the exception of valine (0.59% digestible valine). L-valine was added to the negative control diet to achieve six digestible valine treatments ranging from 0.59 to 0.84% in 0.06% increments. Digestible valine to lysine ratio was determined to be 0.78, 0.77, and 0.74 for body weight gain, total breast meat weight, and total breast meat yield. An optimum ratio was not determined for feed conversion ratio. Corzo et al. (2008) evaluated

dietary valine requirements (total) of Ross × Ross male broilers with three experiments from 0 to 14, 14 to 28, and 28 to 42 d of age and determined the requirements to be 1.00, 0.95, and 0.85%, respectively. Tavernari et al. (2013) examined the digestible valine to lysine ratio in Cobb 500 male broilers from 8 to 21 and 30 to 43 d of age in two independent experiments. In each experiment, seven experimental diets were provided to broilers that consisted of six diets ranging in digestible valine to lysine ratio (0.69 to 0.84 – 8 to 21 d of age; 0.70 to 0.85 – 30 to 43 d of age) and a positive control diet. Optimum valine to lysine ratio for weight gain and feed conversion were determined to be 0.77 and 0.75 and 0.75 and 0.77 from 8 to 21 and 30 to 43 d of age, respectively. Moreover, Berres et al. (2011) ascertained the digestible valine requirement of Cobb × Cobb 500 male broilers from 21 to 42 d of age. Seven digestible valine concentrations were fed ranging from 0.71 to 0.97% in increments of 0.04-0.05%. Based on broken-line methodology, digestible valine requirements were estimated at 0.82 and 0.81%, respectively, for body weight gain and feed conversion ratio. No dietary treatment differences were observed for carcass and breast meat yields. Data from these experiments indicate that the digestible valine to lysine ratio of 0.77 to 0.78 optimizes growth performance and meat yield of broilers.

Isoleucine

Feed-grade valine can be added to diets approximating 0.52 kg/ton without any adverse response in growth performance and breast meat yield of broilers (Corzo et al., 2011). Increasing L-valine supplementation at or beyond 0.78 kg/ton resulted in poor growth performance and meat yield. Optimum ratios of isoleucine, arginine, and tryptophan were not maintained purposely to determine the maximum inclusion of L-valine without supplementing these amino acids. Breast meat yield was adversely affected at L-valine supplementation of 1.04 and 1.30 kg/ton compared with the control-fed birds and this reduction in breast meat yield may be related to a decrease in the isoleucine ratio with the highest inclusion rates of L-valine. Other research has demonstrated that dietary isoleucine can play a key role in breast meat accretion. Kidd et al. (2004) evaluated the dietary isoleucine requirement of male broilers from 42 to 56 d of age. Broilers fed diets containing increasing dietary isoleucine concentrations had quadratic responses for higher breast meat weight ($P \leq 0.001$) and yield ($P \leq 0.001$). Kidd et al. (2000) determined that feeding broilers diets containing a 10% reduction of the isoleucine requirement (NRC, 1994) resulted in a 0.46% reduction in breast meat yield compared with birds fed diets formulated to contain isoleucine at 100% of NRC 1994 recommendations. Dozier et al. (2012) examined interactive effects of digestible valine and isoleucine ratios to lysine on growth performance and meat yields of Ross × Ross 708 male broilers from four to six weeks of age. Increasing the digestible isoleucine to lysine ratio from 0.63 to 0.73 led to a 0.4% higher total breast meat yield (22.0 vs. 21.6%). Rostagno et al. (2011) has reported the digestible isoleucine to lysine ratio as 0.67 and 0.68, respectively, for broilers from 1 to 21 and 22 to 42 d of age for optimum growth performance. In addition, Kidd et al. (2004) determined the total isoleucine requirements to vary between 0.67 to 0.71%, 0.64 to 0.66%, and 0.55 to 0.66%, respectively, from 18 to 30, 30 to 42, and 42 to 56 d of age. The variation in the isoleucine requirement was due to response criteria of interest (body weight gain, feed conversion, and carcass characteristics). Maintaining an adequate isoleucine minimum in diet formulation is needed to optimize growth performance and meat yield of broilers.

Summary

These data indicated that a digestible valine to lysine ratio of 0.76 to 0.78 optimizes growth performance of broilers throughout various phases of production. Dietary valine affects body weight gain and feed conversion more acutely than meat yield responses. Supplementation of L-valine can be used effectively to reduce diet cost with the reduction of soybean meal without compromising broiler performance. In addition, digestible isoleucine to lysine approximates 0.67 to 0.68 should allow for acceptable performance and carcass characteristics. In formulation, it is important to set minimums for valine and isoleucine to avoid a loss in performance and meat yield. This will become very important as the interest increases to formulate lower crude protein diets in broiler production to reduce nitrogen excretion while meeting performance objectives.

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Optimizing Utilization of Co-products in Poultry and Swine Nutrition

Optimiser l'utilisation de coproduits dans l'alimentation des volailles et des porcs

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Abstract

Feed is the single most expensive input for commercial livestock production systems. Thus, profit margins for these systems are negatively impacted when the cost of feed increases drastically, as has been the case in past decade. An approach to mitigating increases in feed cost is to expand the ingredient options that could be used to formulated effective livestock diets, hence the increased interest in the use of co-products. Generally, however, co-products from the seed crushing industry (e.g., canola and flaxseed meal) and ethanol industry (e.g., corn and wheat distiller's dried grains with solubles) contain high levels of dietary fiber and some anti-nutritional factors, which necessitate an accurate determination of chemical composition and nutritive values of these ingredients. The primary goal of diet formulation for poultry and swine is not only to supply nutrients needed to support optimum performance of animals but also to ensure that dietary supply of such nutrients closely matches animal needs. However, because utilization of nutrients in co-products are generally less than those in conventional feed ingredients, accurate diet formulation strategies should be used. Over the years, various diet formulation strategies have been used in poultry and swine diets to supply energy and nutrients. In terms of energy supply, the net energy system and apparent metabolizable energy (nitrogen corrected or not) system have been recommended for swine and poultry diet formulation, respectively. With respect to amino acids (AA), standardized ileal digestible (SID) values are recommended for both species, whereas standardized total tract digestible P and SID P are recommended for swine and poultry, respectively. The determination and use of these coefficients is critical to the optimal utilization of co-products in formulating effective poultry and swine diets, as is the strategic use of feed additives and processing procedures that may enhance energy and nutrient availability in such feedstuffs.

Résumé

Les aliments constituent l'intrant le plus coûteux dans les systèmes commerciaux de production animale. Ainsi, toute augmentation substantielle du coût des aliments, comme celle que nous avons connue au cours de la dernière décennie, a des répercussions négatives sur les marges de profits de ces systèmes. Une façon d'atténuer l'effet de la hausse des coûts d'alimentation est d'élargir la gamme des ingrédients susceptibles d'être utilisés pour la formulation de rations efficaces, d'où l'intérêt accru porté aux coproduits. Cependant, les coproduits de l'industrie de la trituration (p. ex., tourteaux de canola et de lin) et de l'éthanol (p. ex., drêches de distillerie avec

solubles) sont riches en fibres alimentaires et contiennent certains facteurs antinutritionnels, ce qui exige la détermination exacte de la composition chimique et des valeurs nutritives de ces ingrédients. Le principal objectif de la formulation de rations pour les volailles et les porcs est non seulement de fournir les éléments nutritifs nécessaires pour optimiser la performance des animaux, mais aussi de faire en sorte que l'apport en nutriments corresponde le plus possible aux besoins. Toutefois, parce que l'utilisation des nutriments des coproduits est généralement moindre que pour ceux des ingrédients alimentaires classiques, des stratégies de formulation précise de rations devraient être mises en œuvre. Au fil des ans, diverses stratégies de formulation précise ont été utilisées chez les volailles et les porcs pour fournir l'énergie et les nutriments. En ce qui concerne l'apport énergétique, le système de l'énergie nette et le système de l'énergie métabolisable apparente (corrigée ou non pour l'azote) ont été recommandés pour la formulation de rations pour les porcs et les volailles, respectivement. Pour les acides aminés (AA), les valeurs de digestibilité iléale standardisée (DIS) sont recommandées chez les deux espèces, tandis que, pour le phosphore, la digestibilité standardisée pour l'ensemble du tube digestif et la DIS sont recommandées, respectivement chez les porcs et chez les volailles. La détermination et l'utilisation de ces coefficients sont indispensables pour l'utilisation de coproduits dans la formulation de rations efficaces chez les volailles et les porcs, tout comme l'est d'ailleurs l'utilisation stratégique d'additifs alimentaires et de procédés de traitement susceptibles d'accroître la disponibilité de l'énergie et des nutriments contenus dans ces ingrédients alimentaires.

Introduction

The livestock industry in Canada remains under severe competitive pressure. Feed is the single most expensive cost of livestock production. Historically, feed cost has accounted for 65-75% of the total production costs, but it has been increasing due to a combination of poor harvests in different parts of the world, increasing demand for feed grains from the biofuel sector, and speculative buying by funds (Woyengo et al., 2014). To a large extent, energy, amino acids (AA) and phosphorous (P) are the major determinants of the cost of feeding animals. It therefore, makes economic sense, to characterizing feedstuffs on the basis of truly available energy [net energy (NE) for pigs and apparent metabolizable energy (AME) for poultry], standardized ileal digestible (SID) AA and digestible P and possibly price ingredients on such basis. In Canada, canola meal, flaxseed meal, faba beans, wheat co-products, and field peas are the major locally available ingredients and regarded as co-products/alternative feed ingredients for use in poultry and swine diets. Also, there are emerging co-products such as camelina cake and hemp hulls although these ingredients need to be further investigated. Feed cost is and will continue to remain a critical factor determining the profitability of the livestock industry. Thus, enhancing the utilization of co-products in formulating poultry and swine diets offers an effective strategy for controlling feed cost. This will require that such feedstuffs are well investigated in terms of their nutritive value, risks associated with their use (e.g. mycotoxins, anti-nutritional factors), and potential economic benefits when formulated correctly into diets. This paper discusses current diet formulation strategies and the nutritive values of co-products available to the poultry and swine feed industries in Canada. Performance response to feeding diets contain these co-products are also discussed.

Diet Formulations Strategies

A primary goal in formulating livestock diets is not only to supply nutrients needed for optimal performance but also to ensure that dietary supply of such nutrients closely matches animal needs. This is important because nutrients are not only expensive but excessive excretion of some nutrients may cause environmental pollution. Over the years, several diet formulation strategies have been proposed for energy and nutrients supply in poultry and swine diets.

For the past 30 years, the digestible (DE) and metabolizable energy (ME) systems have been widely used in swine diets to supply energy. Although these systems worked well when using high quality ingredients such as corn and soybean meal, it is now clear that they are not suitable for formulating effective diets with co-products that generally tend to contain high levels of dietary fibre. Energy values of diets high in protein and fibre are often overestimated with the DE or ME system (Noblet et al., 1994) resulting in less dietary NE content because the DE and ME systems do not consider the energy lost as heat increment. Therefore, the NE system more precisely predicts the amount of energy used and retained in the pig for fibrous feedstuffs when compared to the DE and ME systems. Consequently, there has been a lot of interest in the determination of the NE contents of various feed ingredients for swine. Indeed, the recently published NRC (2012) has recommended that swine diets should be formulated on an NE system. Compared to swine, AME or nitrogen corrected AME (AME_n) values are widely used to supply energy for poultry (NRC, 1994).

With respect to AA and P supply, the SID and STTD systems are recommended for more accurately matching supply with requirements for achieving more uniform and predictable animal performance (NRC, 2012). These systems have gained more acceptance as several studies have demonstrated that they are additive in a mixture of feed ingredients thus allowing formulation of effective diets based on a wide range of ingredients. For instance,

performance was reduced when pigs were fed diets containing 25% DDGS and formulated on a DE and apparent ileal digestible amino acid basis (Thacker, 2006; Figure 1). However, when diets were formulated on a SID basis, performance and lean meat yield were unaffected by 20% DDGS inclusion (Stein, 2007; Figure 2).

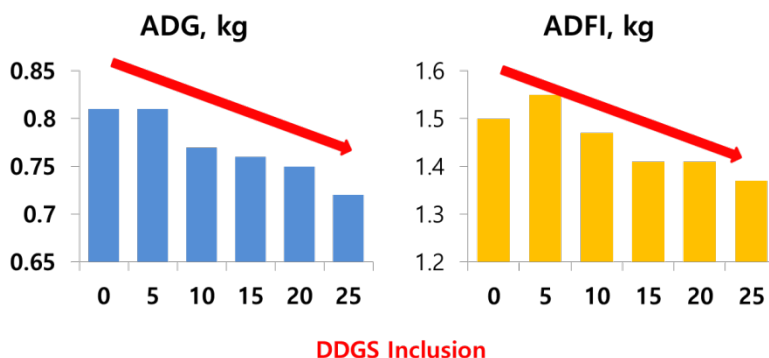


Figure 1. Including increasing amounts of wheat DDGS in diets formulated on a DE and AID amino acid basis reduced performance of growing-finishing pigs (Thacker, 2006).

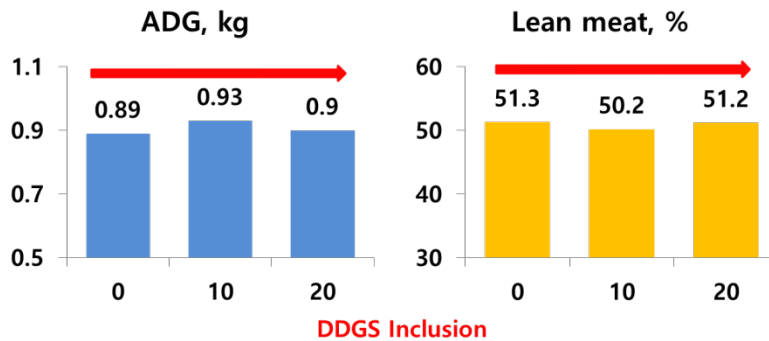


Figure 2. With appropriate diet formulation strategy, higher amounts of wheat DDGS can be fed to growing-finishing pigs with negative effects on performance (Stein, 2007).

Chemical Composition and Nutritive Value of Selected Co-products

Distillers Dried Grains with Solubles (DDGS)

The feeding value of distillers co-products for poultry and swine has been investigated for more than 50 years. The nutritive value of corn-based DDGS for swine has been evaluated extensively (Stein and Shurson, 2009). The increased production of bioethanol from grains, has led to higher prices and increased availability DDGS. The concentration DE and ME in corn DDGS fed to growing pigs were 4,140 and 3,897 kcal/kg of DM, respectively, which are similar to values for corn (Pedersen et al., 2007). In broiler study, AME_n content of corn DDGS was determined to be, on average, 2,971 kcal/kg of DM. Because most starch in grains is converted into ethanol during the fermentation process, other nutrients become highly concentrated (Stein and Shurson, 2009). For example, corn DDGS contains 0.60–0.70% of P with a digestibility of 59%, which is much higher than reported corn (Pedersen et al., 2007). Also, DDGS is a good source of CP and AA because of their high concentrations. The CP concentrations in corn, sorghum, and wheat DDGS are approximately two, three, and four times higher, respectively, than in the respective raw materials. However, DDGS contains a relatively low and variable Lys content and a low SID of Lys due to heat damage during processing (Cromwell et al., 1993). Also, DDGS has a high NSP content, which has 36% insoluble and 6% soluble dietary fiber (Stein and Shurson, 2009). Thus, DDGS should be carefully included in swine and poultry diets to ensure that growth performance is not negatively impacted. Furthermore, high unsaturated fat is a concern in corn DDGS with respect to pork quality (Xu et al., 2010), whereas for wheat and triticale (less fat), high fibre may stimulate gut growth which in turn would reduce the carcass weight (Thacker, 2006). Similarly, high dietary inclusion (>20%) of DDGS resulted in increased polyunsaturated fatty acids (PUFA) content in broiler thigh meat, which may increase tissue oxidation during storage. Therefore, careful inclusion of DDGS should be considered when DDGS are used in diet formulation.

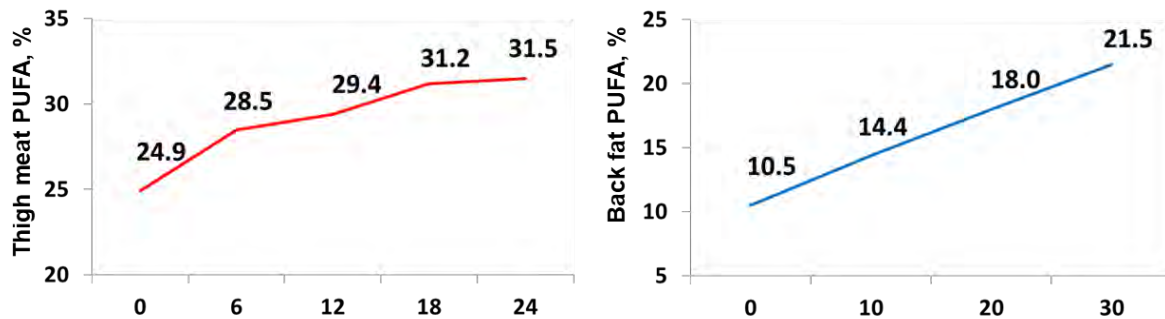


Figure 3. Effects of distillers dried grains with solubles (DDGS) inclusion on polyunsaturated fatty acid content of thigh meat of broilers and backfat of finishing pigs. Data are adapted from Schilling et al. (2010) and Xu et al. (2010).

Canola Meal

Canola meal, a co-product of the seed-crushing industry for oil extraction, is one of the widely used protein source in swine and poultry diets. It is considered a good source of essential AA, especially methionine (Canola Council of Canada, 2015). Solvent extraction is commonly used for oil extraction, resulting in a meal with a residual oil content of less than 5%. Expeller extraction methods are also used to obtain canola oil but are less efficient, resulting in a meal with a greater (8.0 to 15%) residual oil content (Khajali and Slominski, 2012). Thus, expeller-extracted canola meal (EECM) has higher available energy content than solvent-extracted canola meal (SECM). Indeed, AME and AME_n of EECM fed to broiler chickens were 3,039 and 2,649 kcal/kg, respectively, which was 41% and 38% greater than SECM. In pigs the NE content of EECM (2,351 kcal/kg; NRC, 2012) was greater than NE of SECM determined by indirect calorimetry (2,103 kcal/kg; Heo et al., 2014; Kim et al., 2018). Due to the higher processing temperature during solvent extraction compared to expeller extraction, availability of some AA in SECM is reduced (Woyengo et al., 2010a). For example, the SID of Thr and Ser in EECM fed to broiler chickens were greater than for SECM (Woyengo et al., 2010b). The SID of Lys, Met, and Thr in SECM fed to broilers were 77.3, 86.4, and 75.7%, respectively, whereas in EECM the values were 78.7, 83.7, and 83.3%, respectively (Woyengo et al., 2010b). Similarly, the SID of Arg, Ile, Leu, and Phe in EECM fed to growing pigs were greater than in SECM. The SID of Lys, Met, and Thr in SECM fed to growing pigs were 66.6, 84.1, and 72.1%, respectively, and values in EECM were 70.7, 87.4, and 79.5%, respectively (Woyengo et al., 2010b). For finishing pig diets, a key concern with use of EECM is that its high oil content may limit inclusion as unsaturated fat can reduce the degree of saturation in pork fat leading to poor pork quality.

Flaxseed Meal

Flaxseed is a temperate annual crop which is also known as linseed in many countries. Canada is a leading producer of flaxseed, accounting for 30-40% of total world production (Eastwood et al., 2009). The majority of flax in Canada is produced in the prairie provinces of Alberta, Saskatchewan, and Manitoba. Flaxseed is composed of approximately 41% oil, more than 70% of which is polyunsaturated (Eastwood et al., 2009). Flaxseed meal (FSM) is the co-product

remaining after oil extraction from the seed. Its chemical composition varies considerably depending not only on the oil extraction method used but also on variety and growing conditions. Although FSM has a relatively high CP content (30-35%), it characteristically low in Lys content but one of the highest contents of Trp among all feed ingredients currently used in animal nutrition (NRC, 2012). With crystalline Lys being readily available commercially, FSM be effectively utilized in poultry and swine diet formulation.

The use of flaxseed and its related products has gained great attention within the livestock industry due to its oil content and profile. As above mentioned, flaxseed contains approximately 41% oil, most of which is the α -linolenic acid (ALA; omega-3 fatty acid) (Kim et al., 2017b). Although FSM is a co-product after oil extraction, it still contains up to 12% oil, and can therefore still have potential benefits from its fatty acid profile. The actual quantity of oil remaining in FSM varies with the oil extraction methods used. With solvent extraction, the residual oil content does not exceed 3%; however, when expeller-extracted, the meal may contain 8-12% oil. Several studies shown that feeding flaxseed or its products (i.e. meal or oil) increases ALA concentration in animal products (Romans et al., 1995; Enser et al., 2000; García-Rebollar et al., 2008).

Hemp Hulls

Hemp (*Cannabis sativa* L.) has received renewed interest as a profitable agricultural and industrial crop due to its potential health benefits such as high level of dietary fiber and essential fatty acids (Jing et al., 2017; Kim and Nyachoti, 2017). In the past, commercial production of hemp was strictly regulated because of the high concentration of Δ -9 tetrahydrocannabinol, a psychoactive substance found in the hemp plant. However, legal cultivation of industrial hemp under a strict licensing system has been allowed in Canada since 1998 (Health Canada, 2012). Consequently, the availability of HH has increased with increasing cultivation of hemp for human consumption, which presents opportunities for potential use in livestock diets (Kim and Nyachoti, 2017). However, hemp co-products such as hemp oil, meal, cake, and hulls are not currently approved for use in commercial livestock feed in Canada, although on-farm use is permitted. More evidence regarding its efficacy and safety as a feed ingredient for livestock diets is required for approval. In poultry, several studies have investigated the safety and efficacy of hemp meal as a feed ingredient (Gakhar et al., 2012; Neijat et al., 2014; Jing et al., 2017), but more studies are needed for swine. We have reported that HH contain about 21% CP, 19% fat, 0.64% Lys, 0.27% Met, and 0.46% Thr (Table 1). It has relatively high CP and fat contents, meaning that it may be included in diets as a source of energy and protein. However, few studies have determined digestible nutrient contents for poultry and swine. Thus, it is critical that such values are determined to allow effective utilization of this ingredients in poultry and swine diets. Furthermore, high fiber contents (mostly insoluble fiber) needs to be considered when this feed ingredient is included in diets.

Table 1. Chemical composition of corn distillers dried grains with solubles (cDDGS), canola meal (CM), flaxseed meal (FSM), hemp hulls (HH), Dry extruded soybean meal (DESBM), camelina cake (CC), and zero-tannin faba bean (ZT)

Item	cDDGS ¹	CM ¹	FSM ²	HH ³	DESBM ⁴	CC ⁵	ZT ⁶
DM, %	89.4	91.3	92.6	92.4	96.15	92.4	85.4
GE, kcal/kg	4,710	4,332	4931	5,383	4,703	5,046	4,144
CP, %	27.4	37.5	34.2	21.6	42.6	34.7	28.5
Fat, %	8.9	3.2	10.6	19.4	9.5	13.3	1.30
NDF, %	30.5	22.6	41.6	50.7	14.9	40.4	16.0
Ca, %	0.08	0.69	0.41	0.14	0.28	0.31	0.14
P, %	0.60	1.08	0.83	0.58	0.66	0.81	0.42
Lysine	0.90	2.07	1.39	0.64	2.61	1.69	1.85
Methionine	0.57	0.71	0.65	0.27	0.53	0.46	0.22
Threonine	0.99	1.55	1.28	0.46	1.82	1.42	0.97

¹Adapted from NRC (2012).

²Average value from Eastwood et al. (2009) and Kim et al. (2017b).

³Average value from Kim and Nyachoti (2017).

⁴Average value from Opapeju et al. (2006) and Valayudhan et al. (2015).

⁵Average value from Kim et al. (2017a) and Smit and Beltranena (2017).

⁶Adapted from Woyengo and Nyachoti (2012) and Kiarie et al. (2013).

Dry Extruded Soybean Meal

As the production of soybean in Western Canada continues to expand, there has been an increased production of soybean meal products for the livestock feed industry produced through roasting whole beans or through extrusion. A dry extruded and expelled soybean product (DESBM) that has a greater fat content compared to solvent-extracted soybean meal is available to the feed industry (Opapeju et al., 2006). The nutritive value of DESBM has been assessed through performance and apparent nutrient digestibility studies in pigs (Woodworth et al., 2001). In that study, the ME content of DESBM was 15% greater than that of regular SBM, which may result from higher oil content in DESBM compared to regular SBM. Most recently, by indirect calorimetry method, the NE of DESBM was determined to be 2,544 kcal/kg DM (Velayudhan et al., 2015), which is 8% greater than reported NE of regular SBM (2,354 kcal/kg DM) in NRC (2012). Woodworth et al. (2001) reported that apparent ileal digestibility of most AA in DESBM fed to pigs was higher than those in regular SBM. However, the data from our lab has shown that the SID of methionine in DESBM fed to finishing pig was less than that of SBM whereas the SID of isoleucine and leucine in DESBM fed to rooster was less than those of SBM (Opapeju et al., 2006). The contradictory results between two studies might be the result of the age difference of the animals used and species.

Camelina Cake

Camelina (*Camelina sativa*), a member of *Brassica* family, is a fast-growing short-season crop grown in temperate regions for oil production (Kim et al., 2017a). Camelina cake (CC) is a co-

product of oil extraction from camelina and has generally high CP (30%) and fat (10 to 30%) contents. Recently, CC was reported to contain GE, CP and fat contents of 5,046 kcal/kg, 33.2%, and 15.4%, respectively (Kim et al., 2017a). Respective values for Lys, Met, and Thr contents were 1.6, 0.3, and 1.4%. The most abundant indispensable AA were arginine, leucine, lysine, and valine, whereas the sulphur-containing AA (methionine and cysteine) was the least abundant AA. The fat content of CC may be variable because residual oil concentration of the co-product of oilseed is closely associated with the differences in processing conditions. Although the CC may be a good source of protein and energy for swine and poultry diets, there may be a limitation on its utilization due to antinutritional factors such as high glucosinolates concentrations, which may lead to endocrine disturbance and reduced growth performance (Kim et al., 2017a). However, the available energy was not affected by dietary glucosinolates concentration originating from dietary CC, which may indicate that glucosinolates in CC do not have harmful effects on metabolism of animals. Contrary to our observation, increasing inclusion level of CC up to 18% resulted in a 25% decrease in ADG for pigs compared with controls (Smit and Beltranena, 2017). In that study, a linear decrease in feed intake was observed, which imply palatability problems with feeding CC. Bitter taste of CC (mostly due to glucosinolate) cause the negative effects. This suggest that CC does not affect metabolism of pigs, but has negative effects on palatability. Similarly, in broiler chickens a linear reduction in feed intake was observed as dietary inclusion of CC increased up to 10%. Currently, use of CC for animal feed in Canada is only approved for laying hen diets. Therefore, further research is needed to confirm its safety in other livestock species and to identify constituents in CC that are responsible for palatability problems, which could be either selected against by camelina breeders or mitigated through processing.

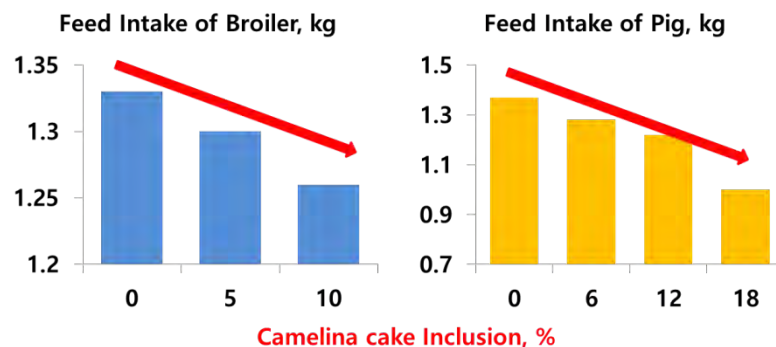


Figure 4. Effects of camelina cake inclusion on feed intake of broilers and pigs. Data are adapted from Ryhanen et al. (2007) and Smit and Beltranena (2017).

Zero-tannin faba beans

Traditionally the use of faba bean in poultry and swine diets has been limited by tannins. However, advancements in crop breeding in Canada and European countries have resulted in the creation of zero-tannin (ZT) faba beans (<1% tannins) (Woyengo and Nyachoti, 2012). With adequate rainfall, seed yield and atmospheric N fixation of ZT faba bean is higher than for field pea (Woyengo and Nyachoti, 2012). Faba bean also has a higher CP compared with field pea (28 vs. 23%). In the recent years, producers in Western Canada have increased the acreage of zero

tannin faba beans, effectively increasing availability for the swine industry (Zijlstra et al., 2004). Extensive research on the effects ZT faba bean inclusion on growth performance has been conducted at the University of Alberta and Prairie Swine Centre (Omogbenigun et al., 2006; Zijlstra et al., 2008). Most recently, University of Manitoba reported nutritive values of ZT fed to poultry and pigs. Overall, results indicate that zero-tannin faba bean may be included in late nursery diets up to a level of 40% without negative effects on weaned pig performance. In growing-finishing pigs, ZT faba bean can fully or partially replace SBM or pea as dietary supplemental protein source without detrimental effects on growth performance, carcass characteristics, and pork quality.

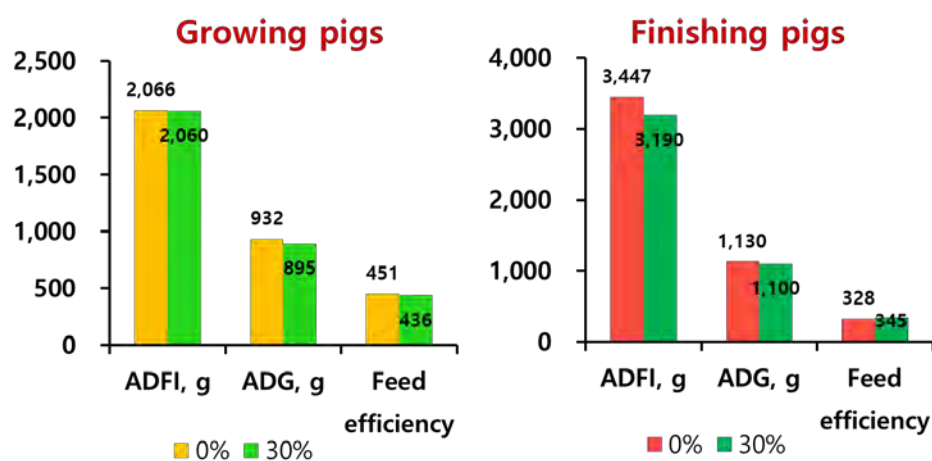


Figure 5. Effects of zero-tannin faba bean inclusion on growth performance of growing and finishing pigs. Data are adapted from Zijlstra et al. (2008) and White et al. (2015).

Implication

There is a wide range of co-products non-traditional ingredients that could be used in the formulation of poultry and swine diets. However, for effective and accurate utilization of these ingredients, it is critical to understand the proportion of their nutrient contents that is digestible/available to the monogastric animal. Currently, digestible AA and P are recommended in swine and poultry, especially for standardized values. For energy, NE and AME are currently recommended for swine and poultry, respectively. Table 2 provides a summary of digestible nutrients and energy in selected co-products that could be used in diet formulations for swine and poultry.

Table 2. Digestibility of nutrients and energy values of selected co-products for poultry and swine

Item	Ingredients						
	cDDGS ¹	CM ²	FSM ³	HH ⁴	DESBM ⁵	CC ⁶	ZT ⁷
Pigs							
DE, kcal/kg	3,620	3,039	3,510	3,186	3,197	3,755	3,276
ME, kcal/kg	3,434	2,849	3,370	2,987	3,128	3,465	3,658
NE, kcal/kg	2,384	2,103	2,430	2,204	2,370	2,383	-
STTD of P, %	65.0	28.0	37.3	22.0	-	53.0	36.0*
SID of Lys, %	61.0	74.2	61.0	-	88.2	57.9	87.1
SID of Met, %	82.0	85.5	65.1	-	82.8	53.4	83.4
SID of Thr, %	71.0	75.5	68.7	-	84.8	52.5	85.8
Poultry							
AME, kcal/kg	2,279	2,005	2,100	-	-	-	-
AME _n , kcal/kg	2,176	1,801	2,070	-	-	1,671	-
SID of Lys, %	73.0	77.3	66.3	-	90.8	76.5	89.9
SID of Met, %	84.9	86.4	66.7	-	92.5	85.5	81.7
SID of Thr, %	73.0	75.7	47.4	-	88.0	72.8	82.2

¹CornDDGS, values are from NRC (2012) ²Canola meal, values are from Woyengo et al., (2010a,b), Trindade Neto et al., (2012), Heo et al., (2014), and Kim et al., (2018a) ³Flaxseed meal, values are from Eastwood et al. (2009), Bandegan et al. (2010), Kim et al. (2017b), and Koo et al. (unpublished) ⁴Hemp hulls, values are from Kim and Nyachoti (2017) and Kim et al., (2018b) ⁵Dry-extruded soy bean meal, values are from Opapeju et al. (2006), Bandegan et al. (2010), and Valayudhan et al (2015) ⁶Camelina cake, values are from Kahindi et al. (2014), Adhikari et al. (2016), Woyengo et al. (2016), and Kim et al., (2017a) ⁷Zero-tannin faba bean, values are from Woyengo and Nyachoti, (2012) and Kiarie et al., (2013).

*Value is adopted from NRC (2012)

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Can Mammary Development be Enhanced by Nutritional Strategies in Swine?

Peut-on se servir de stratégies nutritionnelles pour stimuler le développement mammaire chez le porc?

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Abstract

Sow milk yield is the main determinant of piglet growth rate as it is the only source of energy for suckling piglets. Sows do not produce enough milk to sustain optimal growth of their litter and this problem was made worst with the current use of hyperprolific sow lines. Milk yield is influenced by numerous factors but one factor of importance which is often overlooked is mammary development. In swine, mammary development takes place in prepuberty, as of 3 months of age, during the last third of pregnancy, and during lactation. It is during these periods of rapid mammary growth that nutritional strategies could be used to enhance mammary development. From 90 days of age until puberty, a 20% feed restriction drastically reduces mammary tissue mass whereas decreasing protein intake has no effect on mammary development and feeding the phytoestrogen genistein increases mammary cell number. During late gestation, feeding very high energy levels may have detrimental effects on mammary development and subsequent milk production. Body condition is important to consider. Gilts that have a lower feed intake during gestation and are too thin at the end of gestation have reduced mammary development. Feeding during lactation also affects mammary development. There is an increase in weight of functional mammary glands when sows are fed either more protein or more energy. It is therefore imperative to maximise feed intake of lactating sows. Much remains to be learned about the optimal feeding management to enhance mammary development in gilts and sows.

Résumé

La production laitière de la truie est le principal facteur déterminant la croissance de ses porcelets. Ceci est dû au fait que le lait maternel est la seule source d'énergie pour les porcelets à la mamelle. Les truies ne produisent pas suffisamment de lait pour soutenir une croissance optimale de leur portée, et ce problème s'est aggravé avec l'usage actuel des lignées de truies hyperproliques. La production laitière est influencée par de nombreux facteurs, dont le développement mammaire, un élément important souvent négligé. Chez le porc, la glande mammaire se développe à la prépuberté, soit à partir de l'âge de trois mois, durant le dernier tiers de la gestation, et pendant la lactation. C'est dans ces périodes de croissance mammaire rapide que des stratégies nutritionnelles peuvent être utilisées afin de stimuler le développement mammaire. À partir de 90 jours d'âge jusqu'à la puberté, une restriction alimentaire de 20%

réduit drastiquement la quantité du tissu mammaire, tandis qu'une diminution de l'apport en protéines n'a aucun effet sur le développement mammaire et que la consommation de génistéine, un phytoestrogène, augmente le nombre de cellules mammaires. En fin de gestation, servir des rations à très forte teneur en énergie peut nuire au développement mammaire et à la production laitière subséquente. Il est important de considérer l'état corporel des animaux. Les cochettes dont la consommation alimentaire est moindre durant la gestation et qui sont trop maigres à la fin de la gestation ont un développement mammaire réduit. L'alimentation durant la lactation affecte aussi le développement mammaire. Le poids des glandes mammaires fonctionnelles est plus élevé chez des truies ayant reçu davantage de protéines ou davantage d'énergie. Il est par conséquent impératif de maximiser la consommation alimentaire des truies en lactation. Il reste encore beaucoup à apprendre sur la régie nutritionnelle optimale afin de stimuler le développement mammaire chez les cochettes et les truies.

Introduction

Milk is the main energy source for piglets and is therefore essential for their growth and survival. However, sows cannot produce enough milk to sustain optimal growth of their litters. Indeed, it was shown that ad libitum access to nutrients, achieved via artificial rearing, during the pre-weaning phase results in dramatically heavier weaning weights of piglets compared with sow rearing (Harrell et al., 1993). In that study, artificially-reared pigs weighed 53% more than sow-reared pigs at 21 days of age. More recently, it was also shown that providing supplemental milk to pre-weaning piglets significantly increases their weight at weaning (Miller et al., 2012). The problem of inadequate milk intake by piglets was made even worse with the current use of hyperprolific sows. It is therefore imperative to develop management strategies that will increase sow milk yield. One crucial factor determining sow milking potential is the number of mammary cells that are present at the onset of lactation (Head and Williams, 1991) and more attention should be given to mammary development when developing the best management and nutritional practices for growing replacement gilts and for gestating and lactating sows. Rapid mammary development occurs at three distinctive periods in the life of pigs and it is during these periods that it is possible to attempt to stimulate it via nutritional or other strategies.

When Does Mammary Development Take Place?

At birth, there is relatively little development of the mammary duct system and mammary glands consist mainly of subcutaneous stromal tissue (Hughes and Varley, 1980). Accumulation of mammary tissue and mammary DNA, which is indicative of cell number, is slow until 90 days of age. The rate of accretion of mammary tissue and DNA then increases four- to sixfold (Sorensen et al., 2002) so that by the time the gilt is mated, mammary glands are still very small but contain an extensive duct system with numerous budlike outgrowths (Turner, 1952). Puberty has a stimulatory effect on mammogenesis as parenchymal tissue mass (which contains the milk secreting cells) increases by 51% in gilts that have reached puberty compared with gilts of a similar age that have not started cycling (Farmer et al., 2004).

In pregnant gilts, quantitative development of the mammary glands is slow in the first two-thirds of gestation, while almost all accumulation of mammary tissue takes place in the last third (Hacker and Hill, 1972; Kensinger et al., 1982; Sorensen et al., 2002). Between days 75 and 112 of gestation, mammary glands undergo major histological changes as the adipose and stromal tissues are extensively replaced by the milk-secreting lobuloalveolar tissue (Hacker and Hill, 1972; Kensinger et al., 1982; Ji et al., 2006). Ji et al. (2006) also reported a shift in mammary gland composition going from a high lipid content, reflective of the extensive adipose in the tissue, to a high protein content during the last third of gestation. The location of the gland on the udder affects its development during gestation. The wet weight of middle glands (3rd, 4th and 5th pairs) is greater than that of posterior glands (6th, 7th and 8th pairs) on both days 102 and 112 of gestation (Ji et al., 2006).

Mammary gland development does not stop at the end of gestation but it continues during lactation. The average weight of suckled mammary glands increases by 57% throughout lactation. This increase is linear going from 381 g on day 5 of lactation to 593 g on day 21 (Kim et al., 1999a). Mammary growth in lactation is affected by the position of the gland on the udder. It is greater for the five more anterior teat pairs compared with the more posterior teat pairs (Kim et al., 2000). Mammary development is also altered by parity. Mammary glands from multiparous sows are heavier than those from primiparous sows at the end of lactation but the increase in mammary gland wet weight between day 113 of gestation and day 26 of lactation is greater for primiparous sows, with values of 63, 21 and 39% for sows of parity 1, 2 and 4, respectively (Beyer et al., 1994). Primiparous sows show an increase in both number and size of mammary cells whereas only an increase in cell size is seen in multiparous sows (Manjarin et al., 2011). Interestingly, it was recently shown that what happens during lactation in one parity can affect mammary development in the next parity. When a teat is not suckled in parity one, its development will be significantly reduced in parity two, as well as the growth rate of the piglet suckling it (Farmer et al., 2012c). On the other hand, suckling of a teat for only the first two days of lactation in parity one is enough to ensure that its development the following lactation will not be hindered (Farmer et al., 2017a).

Impacts of Estrogen and Prolactin on Mammary Development

Estrogen

Various hormones are involved in the control of mammary development in swine, the most important ones being estrogens and prolactin. Understanding how these hormones affect mammary development may lead to the development of novel feeding strategies to enhance milk yield. The essential role of estrogens is evidenced by the drastic effect of puberty onset on mammary development (Farmer et al., 2004; Sorensen et al., 2006). During gestation, plasma estrogen concentrations increase dramatically after day 75 (DeHoff et al., 1986) and the drastic increase in metabolic activity of the mammary gland is associated with the increase in estrogens of fetal origin. Indeed, there was a relation between mammary DNA and circulating concentrations of estrogen in sows on day 110 of gestation (Kensinger et al. 1986b,c). An earlier study showed that zearalenone, a mycotoxin with estrogen-like activities, affects mammary development. An increase in mammary glandular elements due to ductal hyperplasia was observed in sows

receiving zearalenone (Chang et al., 1979). Mammary development was even observed in some of the 7-day old piglets sucking the zearalenone treated sows (Chang et al., 1979). Whether this would have beneficial effects on future lactation performance is not known. An attempt was made to specifically stimulate mammary development in gilts by providing a dietary source of estrogen. When 2.3 g/day of the phytoestrogen genistein was added to the standard soya diet of growing gilts from 90 to 183 days of age, there was a 44% increase in mammary parenchymal cells at the end of the treatment period (Farmer et al., 2010a).

Prolactin

Prolactin is the hormone which has received most attention in terms of its effects on mammary development in swine. The first indication that it may affect mammary development came from a trial where prolactin was provided to gilts in an attempt to affect their growth performance (McLaughlin et al., 1997). No measures of mammary composition were made but visual appraisal suggested a stimulatory effect on mammary development. In a later experiment, growing gilts were injected with 4 mg/day of recombinant porcine prolactin for 29 days starting at 75 kg body weight. This treatment led to a 116% increase in mammary parenchymal tissue mass (Farmer and Palin, 2005). However, mammary secretions were present, suggesting premature onset of lacteal secretions. The potential impact of such a development on future milk yield is not known.

As early as 1945, there were indications that consumption of ergotized barley by late-pregnant sows had a detrimental effect on mammary development. Almost no mammary development was present in sows consuming the ergotized barley in late gestation whereas all control sows had a normal mammary development (Nordskog and Clark, 1945). Furthermore, sows fed ergotized barley showed agalactia. A negative impact of ergots on mammary development when fed for 8 days prior to farrowing was also reported more recently (Kopinski et al., 2007). There is a prolactin surge associated with parturition in the sow. It occurs between approximately 2 days prepartum through several days postpartum (Dusza and Krzymowska, 1981). This is most interesting due to the finding that endotoxins have an inhibitory effect on prolactin secretion during the immediate postpartum period, thereby showing a potential relation between suppression of prolactin and insufficient milk yield in sows (Smith and Wagner, 1984). The first demonstration of the essential role of prolactin for mammary development in pregnant gilts was made over 10 years ago using the dopamine agonist bromocriptine to inhibit prolactin secretion (Farmer et al., 2000). When feeding 10 mg of bromocriptine to gilts thrice daily from days 70 until 110 of gestation, mammary parenchymal tissue mass on day 110 of gestation was 581 g compared with 1011 g for control animals, representing a 42.5% decrease.

It was subsequently shown that the specific time-window where prolactin exerts most of its stimulatory effect on mammary gland growth is from 90 to 109 days of gestation (Farmer and Petitclerc, 2003). Feeding 10 mg of bromocriptine thrice daily to gilts during that specific time period decreased total parenchymal mass by 46% on day 110 of gestation but the treatment had no effect when given from days 50 to 69 or days 70 to 89 of gestation. Recent data showed that when creating a hyperprolactinemic state from days 90 to 110 of gestation, using the dopamine antagonist domperidone, there was a significant beneficial effect on secretory activity of mammary parenchyma and on mammary epithelial cell differentiation (VanKlombenbergh et al.,

2013). Subsequent milk yield was also improved on days 14 and 21 of lactation, and piglet weight gain until weaning was increased by 21%. Yet, no measures of mammary composition were obtained.

The timing and degree of stimulation of prolactin secretion may be of importance. King et al. (1996) administered high levels of porcine prolactin to first-litter gilts from day 102 of pregnancy through lactation. While concentrations of RNA and DNA in mammary tissue biopsies were not affected by prolactin administration, milk yield was reduced in treated sows. In another study where recombinant porcine prolactin was administered from days 2 to 23 of lactation in third-parity sows, no significant effects on either milk yield or mammary composition were observed (Farmer et al., 1999). This absence of effect was thought to be due to the fact that mammary receptors for prolactin were already saturated in control animals, thereby preventing the exogenous prolactin from having any biological action (Farmer et al., 1999).

Findings up to date therefore suggest that it would be of interest to find ways to increase circulating prolactin concentrations using feed additives that could be used in commercial swine operations. One possibility was the flavonoid plant extract silymarin (from milk-thistle) which increases prolactin concentrations in rats (Capasso et al., 2009) and increases milk yield in women (Di Pierro et al., 2008) and cows (Tedesco et al., 2004). Yet, a recent study showed that even though silymarin increases prolactin concentrations in gestating sows, this increase is not significant enough to have beneficial effects in terms of mammary development (Farmer et al., 2014a). More specifically, 4 g of silymarin was fed twice daily from 90 days until 110 days of gestation, leading to a 51.8% increase in circulating prolactin concentrations 4 days after the onset of treatment. However, this effect was no longer apparent 15 days later. The absence of beneficial effects on mammary development may be due to the fact that prolactin concentrations were not increased enough or for a long enough period of time. It is possible that a larger dose of silymarin could have had a greater effect. Yet, depending on the required duration of treatment, this would most likely not be economically feasible for producers. Silymarin was also fed to lactating sows with no beneficial effects. When 1 or 8 g/d of silymarin was fed throughout a 20 day-lactation, circulating concentrations of prolactin were not increased and piglet growth was not affected (Farmer et al., 2017b).

Nutritional Impact on Mammary Development

Feeding before puberty

Nutrition of swine in the growing period or during gestation or lactation can affect mammary development. Feed restriction inhibits mammary development as of 90 days of age but not before. Indeed, a 34% feed restriction of growing gilts from 28 days (weaning) to 90 days of age had no significant impact on mammogenesis, whereas a 20% (Farmer et al., 2004) or 26% (Sorensen et al., 2006) feed restriction from 90 days of age until puberty reduced mammary parenchymal mass by 26.3% and 34.2%, respectively. High feeding levels from 90 days of age until puberty are therefore recommended to ensure optimal mammary development of growing gilts. On the other hand, reducing dietary crude protein from 18.7% to 14.4% in that same period does not affect mammary development (Farmer et al., 2004) suggesting that total feed intake is

more important than protein intake per se for mammary development of growing gilts. The impact of feeding flaxseed on mammary development of gilts was investigated because of its high content of secoisolariciresinol diglycoside, which is a precursor for lignin formation, which in turn exhibits estrogenic activities (Adlercreutz et al., 1987). Yet, dietary supplementation with 10% flaxseed from 88 days until 212 days of age did not lead to significant changes in mammary development on day 212 (Farmer et al., 2007b).

There were early indications that diet deprivation followed by overallowance during the growing, finishing, and gestation phases could be beneficial in terms of milk yield and mammary gene expression in swine (Crenshaw et al., 1989). Yet, more recent studies could not reproduce those results. Lyvers-Peffer and Rozeboom (2001) investigated the effects of a growth-altering feeding regimen before puberty on mammary development at the end of gestation. They used dietary fiber (35% ground sunflower hulls) to achieve phases of moderate growth which alternated with phases of maximum growth. They reported that gilts on the moderate feeding regimen from 9 to 12 weeks and 15 to 20 weeks of age had less mammary parenchyma on day 110 of gestation than control gilts. In a later experiment using a similar approach, specific periods of diet deprivation (providing 70% of the protein and DE contents from the control diet) followed by over-allowance (providing 115% of the protein and DE contents from the control diet) in growing gilts did not have any beneficial effect on mammary development after puberty. In fact, this feeding regime led to a decrease in parenchymal tissue mass at puberty (Farmer et al., 2012a). The same nutritional treatment also did not affect parenchymal mass at the end of gestation but led to a tendency for reduced percent protein in mammary parenchyma (Farmer et al., 2012b). It is important to note that in those latter studies by Farmer et al. (2012ab), no compensatory growth was observed in gilts at the end of the finishing period. Even though formulation of the restriction diet decreased the DE content by 30% compared to that of the CTL diet in both Crenshaw and Farmer's studies, the composition of the fiber fraction differed, which may have led to the discrepancy in results. Sunflower hulls were used as fiber source by Crenshaw et al. (1989; J. D. Crenshaw, APC, Inc., Ankeny, IA, personal communication), whereas soybean hulls and wheat middlings were used by Farmer et al. (2012ab). It is therefore still not known whether a compensatory feeding regime in the growing-finishing period only could stimulate mammary development in gilts.

Feeding in gestation

Nutrition during pregnancy does affect mammary development at the end of gestation. An early study where body composition of sows was altered by manipulating protein and energy intakes during gestation demonstrated that overly fat (36 mm backfat) and leaner gilts (24 mm backfat) had similar mammary weights at the end of gestation but that there was a drastic reduction (approximately threefold) in mammary DNA concentration (i.e. cell number) in overly fat gilts (Head and Williams, 1991). Yet, these body conditions are not representative of what is seen commercially and a project was therefore carried out to establish if differences in body fat that are commonly seen can lead to changes in mammary development at the end of gestation. Gilts of similar body weight at mating were fed different amounts of feed throughout gestation (1.30, 1.58 or 1.82 times maintenance requirements) to achieve three levels of body fat, namely, 12-15 (lean), 17-19 (medium) and 21-26 (fat) mm on day 109 of gestation. Parenchymal tissue mass was significantly reduced in lean gilts, being 1059, 1370 and 1444 g for lean, medium and fat

gilts, respectively (Farmer et al., 2016a). It is therefore apparent that being too thin at the end of gestation is detrimental for mammary development. If body condition of gilts differs at mating (innate differences) and these differences are maintained during gestation by varying feeding levels, parenchymal tissue mass at the end of gestation is not affected but its composition is altered (Farmer et al., 2016b). Thinner gilts (12-15 mm backfat) had greater concentrations of protein, DNA (i.e. cell number) and RNA (i.e. metabolic activity) in their mammary parenchyma compared with gilts from the two other groups. Taken together, these studies highlight that feeding during gestation is important in terms of mammary development and that one should avoid underfeeding to ensure maximal amount of parenchymal tissue mass.

Not only amount of feed but also composition of feed during gestation can be important for mammary development. Increasing dietary energy (5.76 vs. 10.5 Mcal/ME) from day 75 of gestation until the end of gestation decreased mammary parenchymal weight and parenchymal DNA on day 105 of gestation (Weldon et al., 1991). On the other hand, increasing protein intake (330 vs. 216 g CP/day) had no effects on mammary development (Weldon et al., 1991). This finding was later corroborated by Kusina et al. (1999) who showed that lysine intakes of 4, 8 or 16 g/day from days 25 to 105 of gestation did not alter mammary development at the end of gestation. When using a period of diet deprivation (providing 70% of the protein and DE contents from the control diet) for the first 10 weeks of gestation, followed by a period of over-allowance (providing 115% of the protein and DE contents from the control diet) until the end of gestation, there was less parenchymal tissue at the end of gestation with no changes in parenchymal tissue composition in treated gilts (Farmer et al., 2014b). The goal of that project was to look at the effect of compensatory feeding on mammary development yet, even though growth rate was increased in the overfeeding period, this increase was not large enough to compensate for the body weight loss in the restriction period during early gestation. A better adapted feeding regime needs to be developed to be able to truly assess the impact of compensatory feeding on mammary development of gestating gilts. A recent report indicated that nutrition of sows in gestation and lactation can affect mammary development of their offspring. Indeed, dietary supplementation with 10% flaxseed from day 63 of gestation until the end of lactation increased mammary parenchymal mass of the offspring at puberty (Farmer and Palin, 2008). This is a first demonstration of such an in utero effect in swine and it leads to new avenues in terms of development of feeding strategies to enhance mammary development.

Feeding in lactation

Nutrition during the last phase of rapid mammary accretion, namely lactation, also affects mammary development, yet there is very little information on the subject. It was shown that both increased energy and protein intakes stimulate mammary gland growth. Indeed, Kim et al. (1999b) fed lactating primiparous sows 4 diets that were a combination of different protein (32 or 65 g lysine/day) and energy (12 or 17.5 Mcal ME/day) levels. Wet and dry weights of suckled mammary glands were positively affected by both energy and protein intakes. Results showed that wet and dry mammary weights were maximized when sows consumed an average of 16.5 Mcal of ME and 950 g of crude protein per day, the latter being equivalent to 52.3 g of lysine daily. It was suggested that lactating sows may need slightly more lysine than the currently recommended amount in order to achieve maximal mammary gland growth during lactation. Litter size positively affects mammary development and it was estimated that lactating sows

should be fed an additional 0.96 g of lysine per day for each additional piglet in a litter of 6 and above (Kim et al., 1999c). It is apparent from published findings that nutrient intake during lactation is important for mammary development, thereby supporting the current recommendation to increase sow feed intake as much as possible during lactation to maximize milk yield.

Conclusion

Mammary development in swine can be altered by many factors, one of them being the nutritional strategy used in the prepubertal, gestation and lactation phases of production. In growing gilts, feed restriction as of 90 days of age hinders mammary development and there are indications that supplying the phytoestrogen genistein or increasing concentrations of prolactin stimulate mammogenesis. Nutritional studies in gestation showed negative effects of overfeeding energy to achieve obesity (36 mm backfat) in late gestation and also showed nefarious effects of a gilt being underfed and having less than 16 mm backfat. When using feed restriction followed by over-feeding in gestation, mammary parenchymal mass was reduced. Yet, the effect of a greater compensatory growth during late gestation on mammary development still needs to be determined. Mammary gland growth in lactation responds to increased protein and energy intakes indicating the importance of nutrient intake for mammary development. Special attention must be given to lysine as lactating sows may need slightly more than currently recommended. Furthermore, litter size must be taken into consideration as it affects mammary development and lysine needs are increased in a litter of 6 or more piglets. It is obvious from our current state of knowledge that much still remains to be learned in order to develop the best nutritional strategies for replacement gilts, gestating gilts and lactating sows in order to maximize their mammary development, hence, their lactation performance.

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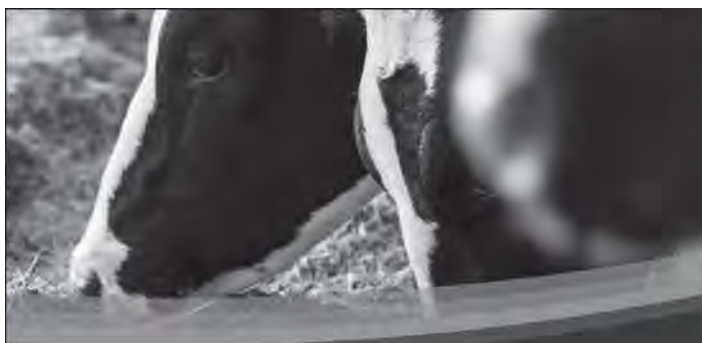
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Growth Performance of Weaned Pigs Fed Raw, Cold-Pelleted, Steam-Pelleted, or Extruded Field Pea

Performance de croissance des porcelets au sevrage recevant du pois de grande culture brut aggloméré à froid, aggloméré à la vapeur ou extrudé

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Abstract

Field pea is an alternative energy and protein source for swine, yet feeding raw field pea to weaned pigs reduced feed efficiency (G:F). Growth performance of weaned pigs fed field pea processed using heat and friction is unknown. To explore, off-grade field pea was ground (4.0-mm sieve), processed via cold-pelleting (70-75°C), steam-pelleting (80-85°C) or extrusion (115°C), then re-ground (3.2-mm sieve). Diets included (per kg) 400 g raw, cold-pelleted, steam-pelleted or extruded field pea replacing 300 g soybean meal (SBM) and 100 g wheat grain and were formulated to 10.0 MJ net energy/kg and 12.1 g standardized ileal digestible lysine/kg. In total, 236 pigs (day 0 body weight: 10 kg; weaned at 20 days of age) were housed in 60 pens in 4 rooms and fed 1 of 5 diets starting 2 weeks post-weaning for 3 weeks. Overall (day 0–21), average daily feed intake (869-878 g/day) of pigs fed raw, cold-pelleted or extruded field pea diets, but not steam-pelleted field pea diet (855 g/day; $P > 0.05$), was greater ($P < 0.05$) than pigs fed SBM diet (807 g/day). Average daily gain did not differ among diets. Thus, G:F (0.61-0.62) of pigs fed raw, cold-pelleted or extruded field pea diets, but not steam-pelleted field pea diet (0.63; $P > 0.05$), was lower ($P < 0.05$) than pigs fed SBM diet (0.67). Final body weight of pigs fed raw, cold-pelleted, steam-pelleted, extruded field pea, or SBM diets were 21.3, 21.2, 21.4, 21.5 and 21.5 kg respectively, and were not affected ($P > 0.05$) by feeding/processing of field pea. To conclude, weaned pigs fed 400 g field pea/kg maintained growth compared to pigs fed SBM. Greater ADFI for field pea diets than SBM diet suggested increasing diet nutrient density may help maintain G:F. Steam-pelleting may be best to utilize low-cost off-grade field pea in weaned pigs to replace 300 g SBM and 100 g wheat grain/kg diet.

Résumé

Le pois de grande culture est une source possible d'énergie et de protéines pour les porcs, quoique son utilisation sous forme brute ait réduit l'efficacité alimentaire chez les porcelets au sevrage (G:A). La performance de croissance des porcelets au sevrage qui ont consommé du pois de grande culture ayant subi un traitement de chaleur et de friction demeure inconnue. Pour remédier à cette situation, du pois de grande culture hors-grade a été moulu (tamis de 4,0 mm), traité par agglomération à froid (70-75 °C), par agglomération à la vapeur (80-85 °C) ou par

extrusion (115 °C), puis moulu de nouveau (tamis de 3,2 mm). Les rations étaient composées de 40 % de pois de grande culture brut aggloméré à froid, aggloméré à la vapeur ou extrudé, au lieu de 30 % de tourteau de soya et 10 % de blé, et ont été formulées de façon à fournir 2,4 Mcal d'énergie nette/kg et 1,2 % de lysine digestible iléale standardisée. Au total, 236 porcs (poids corporel au jour 0 : 10 kg; sevrage à 20 jours) ont été placés dans 60 enclos de quatre salles et, à partir de deux semaines après le sevrage, ont reçu une des cinq rations pendant trois semaines. Globalement, (jours 0 à 21), l'ingestion quotidienne moyenne (869-878 g/jour) des porcs qui ont reçu les rations de pois de grande culture brut aggloméré à froid ou extrudé, mais pas la ration de pois aggloméré à la vapeur (855 g/jour; $P > 0,05$), a été supérieure ($P < 0,05$) à celle des porcs qui ont reçu la ration de tourteau de soya (807 g/jour). Aucune différence de gain moyen quotidien n'a été observée entre les rations. Par conséquent, le ratio G:A (0,61-0,62) des porcs qui ont reçu les rations de pois de grande culture brut aggloméré à froid ou extrudé, mais pas la ration de pois de grande culture aggloméré à la vapeur, (0,63; $P > 0,05$), était inférieur ($P < 0,05$) à celui des porcs qui ont reçu la ration de tourteau de soya (0,67). Les poids corporels finaux des porcs nourris avec les rations de pois de grande culture brut aggloméré à froid, aggloméré à la vapeur, extrudé ou la ration de tourteau de soya étaient respectivement de 21,3, 21,2, 21,4, 21,5 et 21,5 kg et n'ont pas été influencés ($P > 0,05$) par l'utilisation/le traitement du pois de grande culture. En conclusion, les porcelets au sevrage qui ont reçu une ration contenant 40 % de pois de grande culture ont maintenu un rythme de croissance comparable à celui des porcelets nourris au tourteau de soya. L'ingestion quotidienne moyenne supérieure mesurée avec les rations de pois de grande culture par rapport à la ration de tourteau de soya laisse supposer que la plus forte densité de nutriments de la ration pourrait aider à maintenir le ratio G:A. L'agglomération à la vapeur pourrait être le meilleur traitement permettant d'utiliser le pois de grande culture hors-grade peu coûteux chez les porcelets au sevrage pour remplacer 30 % de tourteau de soya et 10 % de blé dans la ration.

Introduction

Field pea (also known as dry peas, *Pisum sativum*) has the largest production volume of all pulse grains in Canada, with 4.8 million MT of dry pea produced in 2016-2017 (AAFC, 2018), largely due to agronomic conditions and pulse grains' increasing role in soil nitrogen fixation and crop rotation. Field pea is a source of energy and amino acids for pigs. Excess production or non-food grade field pea can be included in swine diets to replace soybean meal to reduce feed costs for hog producers (Landero et al, 2014). Field pea contains less crude protein and lysine than soybean meal, more crude protein and amino acids than cereal grains, and more fibre than soybean, corn, or wheat (Woyengo et al., 2014). In comparison to other legume crops, field pea has the greatest net energy value, likely due to its content of digestible starch and fermentable fibre. However, young pigs fed diets containing 600 g raw field pea/kg had reduced growth in the first week and overall 28-day trial despite maintained feed intake (Stein et al, 2010).

Potential reasons for the reduced growth could be due to the presence of anti-nutritional factors, particularly 0.2 to 0.5 mg trypsin inhibitor/g crude protein (Jeziorny et al., 2011). In addition to trypsin inhibitor, lectins and phytate are also present. However, protease inhibitors and lectins are heat labile and may be destroyed by heat. In other pulse grains, extrusion can improve *in vitro* protein and starch digestibility and deactivate trypsin inhibitors, phytic acid, and tannins.

Processing of ingredients may increase nutrient digestibility and growth performance of pigs. However, literature on processing of field pea is scarce and the effects vary. In steam-pelleted diets, inclusion of up to 400 g field pea/kg did not affect feed intake, growth and feed conversion of weaned pigs (Landro et al., 2014). But, extrusion of field pea reduced feed intake of weaned pigs fed diets containing 490 g field pea/kg starting from 2 weeks post-weaning, though diets were not formulated based on net energy (Stein et al, 2010). Thus, a need exists to investigate the effect of processing field pea on nutrient digestibility and growth performance of weaned pigs.

Experimental Design

In total, 236 Hypor crossbred (F1) weanling piglets were used. Piglets were weaned at 3 weeks of age and housed by litter in a nursery room. Selected pigs were randomly assigned to 60 pens in 4 nursery rooms containing 2 gilts and 2 barrows per pen. The study was a complete randomized block design with 5 pens per block, each pen being fed one of the 5 diets. In total, there were 12 pen observations per diet. Pigs began test 2 weeks after weaning and weighed 10.0 kg \pm 0.85 kg and were on test for 3 weeks total. Diets were formulated as followed:

- 1) Soybean meal control (300 g SBM/kg and extra 100 g wheat/kg)
- 2) Raw field pea (400 g field pea/kg)
- 3) Cold-pelleted field pea (400 g field pea/kg)
- 4) Steam-pelleted field pea (400 g field pea/kg)
- 5) Extruded field pea (400 g field pea/kg)

Field pea was sourced from a commercial supplier. All diets were fed as mash and were formulated to 10.0 MJ NE/kg and 12.1 g standardised ileal digestible lysine/kg, and diets were formulated for ideal AA ratio based on table values for field pea nutrient composition. Celite was included as an insoluble marker at 8 g/kg.

Feed intake was measured and individual pigs were weighed weekly and fecal grab samples were collected during the final 2 days of the study. Pen was the experimental unit for statistical analyses of growth performance and digestibility data.

Growth Performance and Digestibility

Dissimilar to data by Landero et al. (2014) and Stein et al. (2010), overall feed intake was greater ($P < 0.05$) for pigs fed diets containing raw, cold-pelleted, or extruded field pea diets (869, 878, and 878 g/d, respectively) than pigs fed the SBM diet (807 g/d), but not for pigs fed steam-pelleted field pea (855 g/d). Results by week yielded different findings. In week 1, feed intake differed ($P < 0.05$) between soybean meal control and extruded field pea, yet in week 3, only feed intake between soybean meal and cold-pelleted field pea differed.

The ADG did not differ ($P > 0.05$) between pigs fed the SBM diet or pigs fed field pea diets at any point in the study. Final BW of pigs fed raw, cold-pelleted, steam-pelleted, or extruded field pea, or SBM diets were 21.3, 21.2, 21.4, 21.5, and 21.5, respectively, and were not affected ($P > 0.05$) by feeding field pea and/or processing. Consequently, overall feed efficiency (d 0-21) was

lower ($P < 0.05$) for pigs fed diets containing raw, cold-pelleted, or extruded field pea (0.61, 0.61, 0.62, respectively) than for pigs fed SBM diet (0.67).

In addition, predicted net energy value of raw, cold-pelleted, steam-pelleted, and extruded field pea diets (9.14, 9.11, 9.15, and 9.09 MJ/kg as-fed, respectively) was lower ($P < 0.05$) than SBM diets (10.02 MJ/kg as-fed). Consequently, the lower net energy value in field pea diets, despite formulation for equal net energy, could explain the difference in feed intake and thus feed efficiency.

Apparent total tract digestibility (ATTD) of dry matter, crude protein, and gross energy differed among diets (Figure 1). The ATTD of dry matter, crude protein and gross energy was greater ($P < 0.05$) for the SBM diet than the field pea diets. Among field pea diets, ATTD of nutrients did not differ ($P > 0.05$).

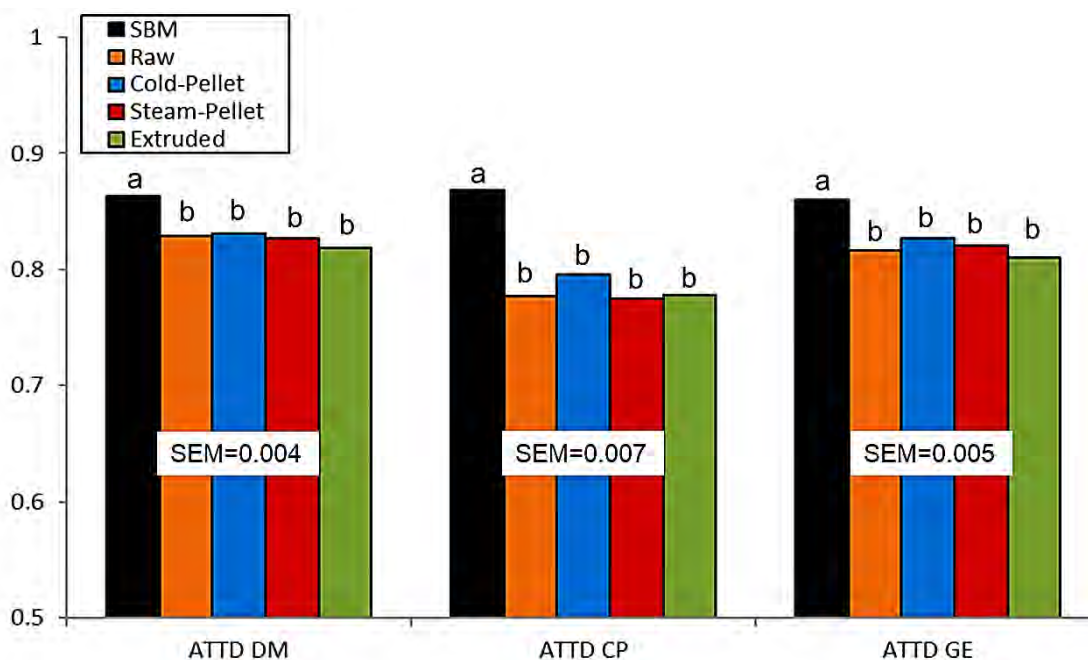


Figure 1. Apparent total tract digestibility coefficients of dry matter, crude protein, and gross energy of weaned pigs fed 400 g/kg raw, cold-pelleted, steam-pelleted, or extruded field pea, or 300 g soybean meal/kg and an extra 100 g wheat grain/kg, for 3 weeks starting 2 weeks post-weaning.

Conclusion

Weaned pigs fed 400 g field pea/kg diet maintained growth compared to pigs fed SBM, providing opportunities to reduce feed cost when the price for field pea is attractive. In phase-3 nursery pig diets, heat processing of field pea might not be required for optimal growth performance.

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Effective Fiber for Dairy Cattle, How Much is Not Enough?

Fibre efficace chez les bovins laitiers, quelle quantité faut-il?

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Abstract

Physically effective fiber (peNDF) is defined as that fraction of NDF that stimulates chewing activity and contributes to the floating mat of large particles in the rumen. A limitation of using peNDF in ration evaluation procedures is that it does not account for differences in rumen fermentability of nutrients, most notably rumen-degraded starch. The physically adjusted fiber (paNDF) system can be used to estimate TMR particle size and diet compositions needed to maintain target rumen conditions. The system is based on equations derived from a meta-analysis and estimates dietary physical and chemical characteristics required to maintain desired rumen conditions in lactating dairy cows. Effective fiber feeding recommendations are based upon diet ADF, NDF, fNDF, starch, proportion of the ration as forage or cottonseed, as well as particle size measures.

Résumé

La fibre physiquement efficace (NDFpe) est définie comme la fraction de la fibre NDF qui stimule la mastication et contribue à la formation du tapis ruminal. Un des facteurs qui limitent l'utilisation de la notion de fibre NDFpe est qu'elle ne tient pas compte des différences dans la fermentescibilité ruminale des nutriments, tout particulièrement de l'amidon dégradé dans le rumen. Récemment, le concept de fibre NDFpe a été réévalué dans le cadre d'un résumé quantitatif de la littérature disponible identifiant les caractéristiques physiques et chimiques des rations totales et établissant des liens entre ces caractéristiques et la consommation alimentaire, le comportement de mastication et la fermentation ruminale. Ce système de fibre physiquement corrigée (paNDF) peut être utilisé pour estimer la taille des particules des RTM et la composition des rations permettant de maintenir les conditions souhaitées dans le rumen. L'objectif de cet article est de donner une mise à jour sur les connaissances associées à la fibre efficace et aussi de décrire le système paNDF dans le contexte de son application à la ferme.

Introduction

The physical nature of fiber consumed by the dairy cow is known to affect feed intake, chewing activities, rumen fermentation and ultimately milk production and composition. In fact, because dairy cattle are grass and roughage eaters (Hofmann, 1989) and it is generally well understood that cows require coarse roughage and that this is “effective” in maintaining normal rumen fermentation, function, and overall health (Clark and Armentano, 1993). With this being established, a number of investigators have sought to develop methods to quantitatively measure coarseness of roughage and integrate these measures into general feeding recommendations (Santini et al., 1983; Mertens, 1997). Probably the most well-known measure is physically effective fiber (**peNDF**) which is defined as that fraction of NDF that stimulates chewing activity and contributes to the floating mat of large particles in the rumen (Mertens, 1997). It was proposed that peNDF of individual feedstuffs could be estimated by multiplying a chemical measure of fiber in a feed by a physical measure. Over the last 20 years the Penn State Particle Separator (**PSPS**) has been widely used on-farm to measure the particle size TMR (Lammers et al., 1996; Heinrichs and Kononoff, 2002). Additionally, researchers have used the PSPS to report the physical characteristics of both forages and TMR in peer review scientific publications. Although it has been proposed that particle size measures using the PSPS could be used to estimate peNDF (Zebeli et al., 2012) such application is not widespread. Recently, the concept of peNDF has been re-evaluated by quantitatively summarizing available literature reporting physical and chemical characteristics of total diets and deriving equations that relate these to feed intake, chewing behavior, and ruminal fermentation (White et al., 2017a). This physically adjusted fiber (**paNDF**) system can be used to estimate TMR particle size and diet compositions needed to maintain target rumen conditions. The objective of this paper is to provide an update on findings related to effective fiber and to also outline the paNDF system for on-farm application.

New Method to assess effective fiber

Feeding diets low in effective fiber may precipitate and contribute to the cascade of factors associated with ruminal acidosis; but the interactive effects of dry matter intake, digestibility, nonstructural carbohydrate levels, should also be considered. Unfortunately, in many studies it is difficult to draw a clear link between peNDF and rumen pH. This is often the case when peNDF is decreased as grain is added to the diet. In this case, particle size is reduced but the portion of readily digestible carbohydrate is also increased. Here rumen pH is almost always reduced but this may be a function of reduced saliva flow and increased organic acid production with the latter often having the greatest effect. Thus a limitation of using peNDF is that it does not account for differences in rumen fermentability of nutrients, most notably rumen-degraded starch (Dijkstra et al., 2012). Feeding recommendations of carbohydrates of NRC (2001) were summarized in a simple table (see Table 4-3, Page 37 from that source). In this table minimum concentrations of forage NDF (**fNDF**), NDF, and non-fiber carbohydrates could be determined through interpolation. This table has proven to be extremely useful, but it did not include starch and also did not offer any recommendations regarding the particle size of a TMR. In fact, the table caption specified that these recommendations assumed that the forage particle size was adequate. In addressing this void, it has been suggested that the peNDF index is an

oversimplification (Plaizier et al., 2008) of a complex phenomenon. With this in mind, we evaluated different peNDF representations as some particle size measure multiplied by diet NDF content and concluded, that despite the fact that this product does account for some variation in ruminal pH keeping, these dietary factors should be separated as core components and this will allow for consideration of other dietary components that influence pH (White et al., 2017a). We further hypothesized that the utility of peNDF could be expanded and improved by dissociating NDF and particle size and considering other dietary factors, all integrated into a system that can be used to estimate minimum particle sizes of TMR and diet compositions needed to maintain ruminal pH targets (White et al., 2017b). The system is based on equations derived from a meta-analysis (White et al., 2017a) and estimates dietary physical and chemical characteristics required to maintain desired rumen conditions in lactating dairy cows. In practice, the paNDF system generates feeding recommendations for diet characteristics that are based upon computation. All particle size measures used in the paNDF system are determined with PSPS and on a DM basis.

Modeling “rumen conditions” with ensemble models

Accurately modeling the rumen environment is challenging for several reasons. First, rumen conditions are difficult to measure and report; and this leads to uncertainty (Sarhan and Beauchemin, 2015). Second, it is difficult to identify or build datasets that possess sufficient independent variation within independent variables. This may make derivation of useful parameters estimates somewhat problematic. In practice, no single study can possibly evaluate all of dimensions simultaneously. The challenge of accurately modeling and predicting “conditions” is also existent in the field of weather forecasting (Meier et al., 2014). To overcome these challenges some climatologists employ what is known as “*ensemble modeling*” and use the approach to generate predictions of weather patterns as affected by various driving forces (Meier et al., 2014). We chose to use this multi-dimensional approach to predict dietary physical and chemical characteristics required to maintain desired rumen conditions (White et al., 2017a). Our target for prediction of the desired “rumen conditions” was mean ruminal pH. Ruminal pH was chosen because it was frequently reported, in many of the studies included in our dataset, but it should be noted that other measures that were rarely reported such as minimum or maximum pH and time under some specific pH may better represent risk of acidosis. As already mentioned this paNDF system that can be used to estimate TMR particle size and diet compositions needed to maintain target rumen conditions.

Structure of the ensemble

An ensemble modeling approach is used to generate means and confidence intervals to describe the need for particle size, fiber, and other dietary components in diets for lactating cows. In this approach a “mixture of expert” (MEx) models from a range of dietary scenarios, such as high or low starch diets, are identified and rumen pH is then predicted with each expert model individually (Figure 1). The mean of the predicted pH is estimated based on dietary composition using expert algorithms. A confidence range is then estimated based on the minimum and maximum predictions of the ensemble. In practice, an ensemble of models aggregates predictions

from multiple different models (Table 1) to yield a mean and range of responses. Compared with individual models, ensembles may provide more reliable predictions of events, estimate confidence in the reliability of those predictions, and are less likely to generate systematic errors. For example, rather than forcing integration of all models over an entire range of conditions such that the full range has areas of instability, the ensemble approach integrates equations with varying weighting factors over the entire range of conditions. Compared with individual models, an ensemble approach has improved utility, particularly in situations where minimal data is available for equation development. As illustrated in Figure 1, the individual expert models correspond to “model 1, model 2, model 3,” with each model being selected as an “expert” based on its performance against subsets of the data. In our case, the available input data were then run through each model, resulting in 6 predictions of pH. An algorithm was then used to consolidate those 6 predictions into a single pH prediction. The predicted pH was then back-calculated and a recommendation of material to be retained on the 8 mm sieve of the PSPS is generated.

Rumination activity and rumen pH

A general concept related to physically effective fiber is that coarse fiber particles stimulate chewing activity and this in turn, stimulates saliva production that buffers the ruminal environment (Beauchemin et al., 2008). Although total chewing time, as the sum of time eating and ruminating, is commonly reported in studies which have evaluated effective fiber, in our study the effects of total chewing time, as well as eating time, ruminating time (and these factors divided by DMI) on rumen pH was evaluated. Interestingly, of all of the chewing activities tested, only rumination time per unit of DMI was observed to significantly affect rumen pH (Table 1). In general compared to time spent eating, the time spent ruminating likely has a greater influence on rumen pH (Zebeli et al., 2010) and has been observed experimentally (Beauchemin et al., 2003). This may be because cows spend as much as twice the amount of time ruminating than eating and more saliva is produced from rumination activities. (Maekawa et al., 2002a, b). The importance of rumination is not only limited to saliva production and rumen pH as, the act of ruminating is also believed to be closely integrated with reticulo-ruminal motility and consequently overall gut health (Van Soest, 1994). The equation used to predict rumination time is listed in Table 1 and factors observed to affect it included particle size measures of the TMR, NDF and starch content of the TMR, and DMI.

The objective of creating the paNDF system was NOT to develop a predictive equation of rumen pH but to use it as a target for of desirable rumen conditions. Ruminal pH is known as a key physicochemical measure of rumen fermentation (Aschenbach et al., 2011a; Penner et al., 2011). If too low, it can negatively affect rumen microbes and inhibit fiber digestion (Krajcarski-Hunt et al., 2002) and also the flow of microbial CP out of the rumen (Firkins, 1996, 2010; Russell and Wilson, 1996). In our ensemble approach two different models were used to predict rumen pH (Table 1). As the consumption of starch leads to the increased production of organic acids (Firkins, 1996), it was not surprising that starch was used in both models to predict pH. Currently, there is not agreement for the “best or optimal ruminal pH” for lactating dairy cows but White et al. (2017b) used 6.1 as an example. Overall, our quantitative findings provides a comprehensive approach to estimating the effective fiber needs of dairy cattle as both TMR particle size and diet NDF influence both DMI and rumination time and this is in agreement with

mechanistic expectations, and these factors in turn were integrated into a system that could be robustly related to observed ruminal pH in dairy cattle.

Forage fragility, a new consideration

Fragility of a feed has been defined as the rate at which plant tissues contained in a feed particle are further fragmented into small particles (Grant, 2010). Compared to fiber in grasses, the fiber in legumes is thought to be more fragile and can be more easily fragmented (Kammes and Allen, 2012). Consequently, legumes stimulate less rumination and in turn, salivary buffer production. Grasses also have a higher content of hemicellulose (Van Soest, 1994), which crosslinks with lignin, may be less fragile, and might be more effective in stimulating chewing activity (Mertens, 1997). In an attempt to account for this, we included ADF/NDF as an indirect measure of forage fragility. A laboratory method to measure forage fragility has been developed (Farmer et al., 2014), but it is not widely used either in the field or in published studies.

Towards on farm application of paNDF

Figure 2 illustrates how inputs are used to generate feeding recommendations for target rumen conditions. In this figure the proportion of TMR on the top screen (19-mm) is varied on the top axis by 6, 12 or 18 % while fNDF varies on the bottom axis, starch varies on the right axis, and the model solves for the left axis which is the proportion of TMR on the second screen (8-mm). In the top left graph of this figure, depicting 6% of TMR DM retained on a 19-mm screen and 15% TMR DM starch, two inflection points are visible. One occurs at approximately 16.0% fNDF; and the other, at approximately 26.5% fNDF. This figure can be interpreted to suggest that ruminal pH can be maintained in a diet low in fNDF (16.0 %) by increasing the proportion of TMR (between 40 and 60%) retained on the 8-mm sieve. Alternatively, when feeding a diet high in fNDF (26.6%), a lower proportion of TMR (< 20 %) retained on the 8-mm sieve is needed. In practice, a true recommendation for the percentage of DM material on the 8-mm sieve should be based on the diet target fNDF and likely lies somewhere between these 2 inflection points. An additional example can be found in the figure depicting 6% of TMR DM retained on a 19-mm screen and 25% TMR DM starch, in which one inflection point at approximately 22% fNDF is visible. This figure can be interpreted to suggest that longer TMR particles plays a lesser of a role in maintaining pH when fNDF is greater than 22 %.

Deriving solutions or feeding recommendations with the paNDF system a mobile phone application will be available free of charge early in 2018. To use the application users will simply key in desired rumen conditions, diet ADF, NDF, fNDF, starch, proportion of the ration as forage and cottonseed as well as particle size measures. Users can then use the application to determine the proportion of TMR that should be retained on the second sieve (8-mm) of the PSPS to maintain a defined rumen pH. The application will also provide a confidence interval for all recommendations. It should be stressed that meeting the derived feeding recommendations will not guarantee a specific average rumen pH in the herd. The application was designed to generally predict rumen conditions as affects by major diet factors. Other factors are known to affect rumen pH and could not be included in the system. These include the concentration of

other carbohydrates such as water-soluble carbohydrates, and soluble fiber (Hall et al., 1999); chemical or physical processing of feed (Firkins et al., 2001); use of ionophores (Firkins and Yu, 2015); feeding management and behavior (Miller-Cushon and DeVries, 2010), associative rumen effects such as volatile fatty acid and ammonia absorption and urea secretion in rumen (Aschenbach et al., 2011b); and dietary cation-anion difference (Iwaniuk and Erdman, 2015).

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Table 1. Models developed by White et al. (2017a) through during ensemble model training (units of all parameters on a DM basis) and used to generate feeding recommendations for effective fiber (adapted from Whit et al., 2017b).

Response	Equation ¹
DMI, kg/d	$-0.889 - 0.460 \times MPS + 0.0203 \times BW + 0.110 \times Forage + 0.794 \times NDF - 0.0117 \times (NDF \times NDF)$
	$-1.74 - 0.432 \times MPS + 0.0218 \times BW + 0.163 \times Cottonseed + 0.117 \times Forage - 0.238 \times fNDF + 0.771 \times NDF - 0.0116 \times (NDF \times NDF)$
Rumination Time, min/d	$-357 - 16.7 \times MPS + 4.34 \times 19mm + 2.49 \times 8mm + 71.5 \times DMI - 1.54 \times (DMI \times DMI) + 4.78 \times NDF - 1.68 \times dNDF - 2.35 \times dStarch$
pH	$12.0 + 0.0112 \times fNDF - 0.0190 \times Starch + 0.0003448 \times (Starch \times Starch) - 0.679 \times CP + 0.0186 \times (CP \times CP) + 0.01052 \times (Rumination\ Time/DMI)$
	$6.72 + 0.0137 \times fNDF + 0.00798 \times Starch - 0.0456 \times CP - 0.00835 \times dStarch + 0.0204 \times (Rumination\ Time/DMI)$

¹ MPS, estimated mean particle size from PSPS data in mm; BW, body weight in kg; All dietary concentrations are on a DM basis: Forage, % of forage in the TMR; NDF, % NDF in the TMR; Cottonseed, % of cottonseed in the TMR; fNDF, % of forage NDF in the TMR; 19-mm, % of TMR retained on the 19-mm sieve of the PSPS; 8-mm, % of TMR retained on the 8-mm sieve of the PSPS; DMI, dry matter intake, kg/d; dNDF, rumen degraded NDF as estimated by White et al., 2017; dStarch, rumen degraded starch as estimated by (White et al., 2016); Starch, % of starch in the TMR; CP, % of CP in the TMR; Rumination time, time spend ruminating, min/d.

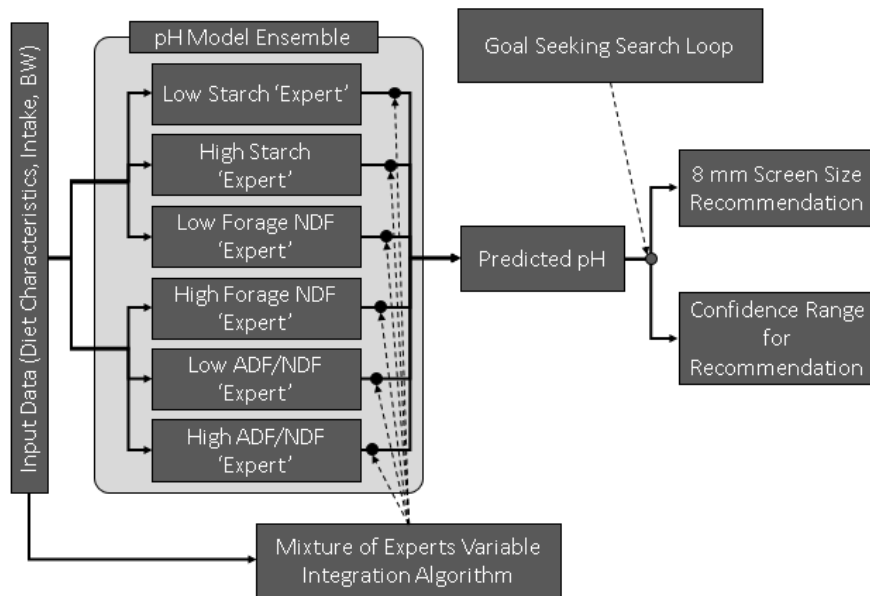


Figure 1. Depiction of strategy to estimate mean and confidence range of pH responses estimated by the model ensemble. Various "expert" models are identified (high starch vs. low starch) and pH is estimated with all expert models individually. The weighted mean of the predicted pH from 6 equations is estimated based on dietary composition using the variable mixture of experts integration algorithm. The confidence range is estimated based on the minimum and maximum predictions of the ensemble (adapted from White et al., 2017b).

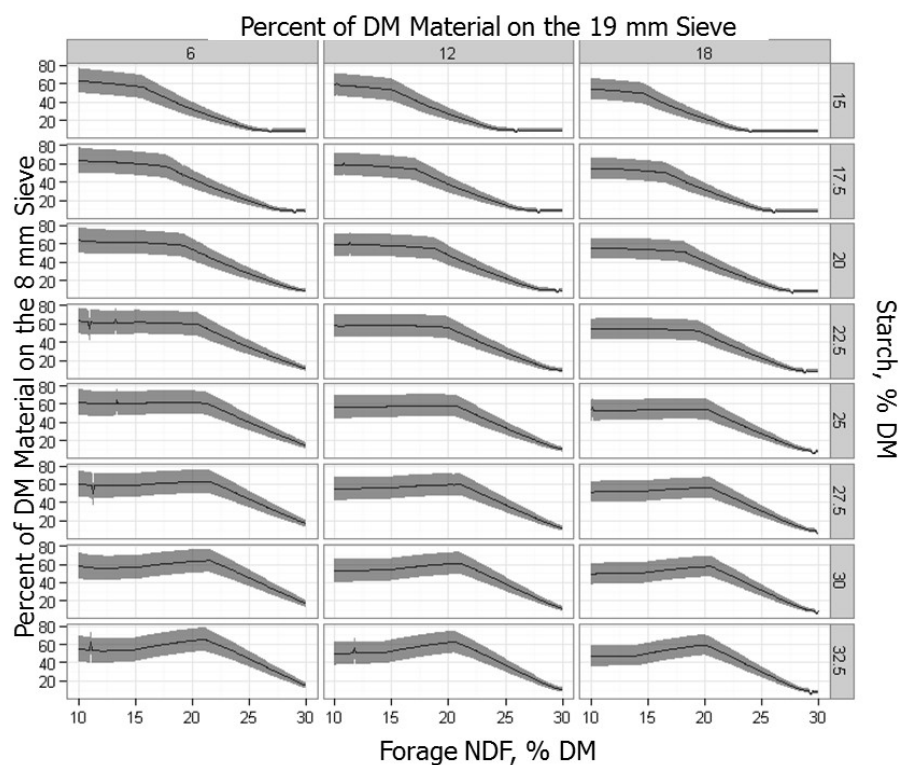


Figure 2. Response surfaces generated by the multi-model ensemble for a target pH of 6.1. Curves were generated by iterating through the system of equations (adapted from White et al., 2017b).

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Abstract

On many dairy operations, transition cow disorders account for over half of mature animal health problems. Impaired immune function and a pro-inflammatory state coincide at the start of lactation in many cows, and are linked to increased risk of transition disorders. A growing number of feed and pharmaceutical products are offering a variety of means to attempt to enhance immune function in the transition period, and other tools are being tested for limiting inflammation. Inherent links between inflammation and immunity raise important questions about whether dairies can “have their cake and eat it, too”, by improving immunity while avoiding inflammatory conditions. These questions are still being resolved; however, several studies suggest that net benefits on health and productivity can be achieved. Administration of anti-inflammatory drugs at the beginning of the lactation have substantially increased whole-lactation milk yield while improving retention in the herd. Likewise, feeding immunomodulatory feed additives have enhanced immune response while increasing milk yield, in some cases. Transition cow strategies have the potential to offer outstanding returns on investment, because of the potential to enhance cow retention and because of long-term carryover benefits on milk production. In summary, although we just beginning to understand altered immune function, inflammation, and their intersection in the transition dairy cow, there are early hints that tweaking these systems can sometimes dramatically improve health and productivity.

Résumé

Dans beaucoup d'exploitations laitières, les affections dont souffrent les vaches en transition comptent pour plus de la moitié des problèmes de santé rencontrés chez les animaux adultes. Chez de nombreuses vaches laitières, le début de la lactation est marqué à la fois par l'altération de la fonction immunitaire et par un état pro inflammatoire, phénomènes associés à une augmentation des risques de désordres pendant la transition. Un nombre grandissant de produits alimentaires et pharmaceutiques offrent divers moyens pour tenter d'améliorer la fonction immunitaire durant la période de transition, et d'autres outils pour limiter l'inflammation sont présentement à l'étude. Les liens essentiels entre l'inflammation et l'immunité nous forcent à se demander si les exploitations laitières peuvent « avoir le beurre et l'argent du beurre », c'est-à-dire s'il est possible d'améliorer l'immunité tout en évitant les conditions inflammatoires. Ces questions ne sont toujours pas résolues; cependant, plusieurs études suggèrent la possibilité d'obtenir des avantages nets pour la santé et la productivité. Une augmentation substantielle du rendement en lait pour l'ensemble de la lactation et une plus longue durée de vie productive dans le troupeau ont été associées à l'administration de médicaments anti-inflammatoires en début de lactation. De même, l'utilisation d'additifs alimentaires immunomodulateurs a stimulé la réponse immunitaire tout en augmentant le rendement en lait, dans certains cas. Les stratégies applicables aux vaches en transition présentent un remarquable potentiel de rentabilité, en raison des possibilités qu'elles offrent d'allonger la durée de vie productive des vaches et de leurs avantages différés à long terme sur la production laitière. En résumé, bien que nous commençons à peine à comprendre les mécanismes qui interviennent dans l'altération de la fonction immunitaire, l'inflammation et leur point de rencontre chez la vache laitière en transition, nous percevons certains signes qui nous laissent croire qu'en modifiant légèrement ces systèmes, il est parfois possible d'obtenir un effet spectaculaire sur la santé et la productivité.

Why Worry About Immunity?

Large-scale analysis of dairy herd records suggests that, around the globe, transition cow problems account for over half of mature animal health problems on a typical dairy farm. There are some obvious risks to cows immediately after calving, including the potential for latent mastitis cases to re-emerge at the onset of lactation and the tissue trauma from calving. However, there is also a well-documented alteration in immune function during the weeks around calving (Kerhli, 2015). In particular, the function of innate immune cells seems to be consistently impaired. Innate immune cells are those involved in quick recognition and clearance of pathogens, independent of pathogen-specific memory (e.g., antibodies).

Why is the immune system of transition cows suppressed? The exact reasons for decreased immune function during the transition period are complex. However, studies with mastectomized cows suggest that the primary driver is not gestation and calving, but rather lactation and the metabolic changes that come with it (Nonnecke et al., 2003). Numerous large studies have demonstrated that metabolic diseases (e.g., ketosis) put cows at higher risk of contracting clinical infections; likewise, cows with infectious diseases (e.g., metritis) are also at higher risk of subsequent metabolic disorders. The inter-dependent nature of the immune and metabolic systems in the animal are only now becoming clear, but high blood ketone and non-esterified

fatty acid concentrations as well as hypocalcemia are known to limit the responsiveness of immune cells to pathogenic signals. Cows with excessive body condition experience more dramatic drops in immune function at calving, possibly as a consequence of oxidative stress. As a result, nutrition of the transition cow can have a large influence on immunity during this time, even beyond the vitamins and minerals that have received focus in the past.

There is some direct evidence that poor immune responsiveness in the transition period is predictive for incidence of infections during this time. In one study, 5 of 31 cows were identified as poor immune responders 4 weeks before calving. All 5 of these cows developed clinical infections during the first 2 months of lactation, whereas only 3 of the other 26 cows did so (Catalani et al., 2013). A much larger study of 458 Holstein cows demonstrated that a measure of antibody-mediated immunity was highly predictive of mastitis incidence, with the top quintile showing a 42% lower mastitis incidence than herdmates (Thompson-Crispi et al., 2013). It is likely that the high rate of infections in early lactation can be attributed in part to immunosuppression.

What Does Inflammation Have to Do with Transition Cows?

Inflammation is a key component of the immune response to infection or tissue damage. Immune cells that first sense pathogens or signs of traumatized cells release signals that activate pain sensors, promote blood flow to the local tissue, and cause fever, accounting for the traditional signs of inflammation. Additionally, the systemic effects of inflammation include an alteration of liver function, typically called the acute phase response. Most of these responses are beneficial for recruiting innate immune cells to the site of immune activation and for inhibition of bacterial growth, but they come at a cost to the animal. Importantly, inflammation can occur in the absence of a true pathogen challenge and can occur without the traditional signs of focal pain, swelling, and redness. When blood markers of inflammation are elevated in the absence of clinical signs, it is often referred to as sub-acute inflammation.

The presence of an acute phase response in postpartum dairy cows is well-established (Bradford et al., 2015). Although early studies focused on associations between inflammatory markers and diseases such as mastitis and metritis, numerous studies in the past decade have demonstrated that inflammatory and acute-phase mediators are elevated in the days after parturition, even in cows that are apparently healthy. This growing body of evidence suggests that either the processes of parturition and galactopoiesis induce inflammation directly or that infections or endotoxin affect far more fresh cows than is currently recognized. Whatever the explanation, the prevalence of post-calving inflammation raises important questions about the implications for early lactation cows.

Although most transition dairy cows apparently experience a period of inflammation, the magnitude of this inflammatory condition varies greatly between cows. Bertoni et al. (2008) assessed the importance of this variation by measuring a panel of inflammatory markers and separating transition cows into quartiles for degree of inflammation. Cows in the highest quartile had significantly lower milk yields than those in the lowest quartile throughout the first month of lactation, differing by 20% on day 28 of lactation (Bertoni et al., 2008). One metric that has been

used in this respect is paraoxonase, a plasma biomarker that is potently suppressed by a variety of inflammatory stimuli. Transition cows with high paraoxonase concentrations, in addition to having lower concentrations of acute phase proteins and reactive oxygen metabolites, produced 1,971 L more milk (24%) over 305 days than those in the lowest quartile for paraoxonase (Bionaz et al., 2007). Other findings suggest that stronger inflammatory responses in the first week of lactation are associated with decreased whole-lactation milk yield (Huzzey et al., 2015). Plasma concentrations of haptoglobin (an acute phase protein) greater than 1.1 g/L were associated with a 947 L decrease in 305-day mature equivalent milk yield, and elevated haptoglobin was also associated with a 19% decreased risk of conception. Abnormally high markers of inflammation are associated with poor production, health, and fertility outcomes.

Responses to Anti-Inflammatory Treatments

Motivated by evidence linking early lactation inflammation to decreased health and productivity, we conducted a study with 78 cows assigned to either control or sodium salicylate delivered via drinking water (2 g/L) for the first 7 days of lactation (Farney et al., 2013). Sodium salicylate is a member of the non-steroidal anti-inflammatory drug (NSAID) class, and is the parent compound of aspirin. At first the results did not look very promising, with no improvement in metabolic health and no increase in early milk yield. However, as lactation progressed, the oldest cohort of cows treated with salicylate (those in parity 3 and greater) responded by producing 21% more milk over the full lactation, and fully 30% more milk fat, than parity-matched controls. On the other hand, primiparous cows treated with salicylate tended to produce less milk, suggesting a potential parity difference in either baseline inflammatory status or response to inflammatory signals.

We subsequently completed a follow-up study to evaluate whether postpartum treatment of multiparous cows could increase whole-lactation productivity of cows on a commercial farm. To facilitate treatment in a commercial setting, we shortened postpartum treatment to 3 days (sodium salicylate) or 1 day (meloxicam) and compared them to placebo treatments (Carpenter et al., 2016) across 153 cows. Despite this very limited treatment window, cows treated with either NSAID produced about 10% more milk over the whole lactation compared to placebo. Over the 365 days following treatment, meloxicam also tended to delay removal from the herd based on survival analysis ($P = 0.06$; 30, 35, and 38 of 51 cows remained at 365 d postpartum for control, salicylate, and meloxicam, respectively). Meloxicam primarily affected early-lactation culling, and health records recorded by the farm suggested that metabolic disorders accounted for most of this decrease.

Several other groups in a variety of countries have failed to observe significant impacts of postpartum anti-inflammatory treatment on milk yield, and it remains to be seen whether a treatment paradigm can be found that is consistently effective. However, we believe that impacts on long-term milk yield likely require treatment relatively early after calving (though not before the placenta is cleared); that treatment responsiveness is not limited to cows with calving difficulties; and that milk yield must be monitored for at least 60 days into lactation to have a good chance to observe the impact of anti-inflammatory treatment.

The use of anti-inflammatory drugs to treat nonspecific postpartum inflammation is not currently approved. Therefore, it is worthwhile to consider whether some feed ingredients might offer the same anti-inflammatory benefits without the use of regulated pharmaceuticals.

Polyphenols are a diverse class of compounds found in nearly all plants in varying concentrations. Some polyphenols have been clearly shown to have anti-inflammatory effects, and a recent study demonstrated some exciting responses in dairy cattle during the transition period. Winkler et al. (2015) supplemented cows with a feed supplement containing green tea and curcuma extract (both potent sources of polyphenols) for the close-up period through 9 weeks in milk. In addition to decreasing liver lipids, supplementation decreased plasma NEFA concentrations after calving and increased milk yield by approximately 4.5 L/day in weeks 4 – 8 of lactation. It is not entirely clear that these production responses were due to anti-inflammatory effects of the polyphenols, but they certainly warrant further study.

A different nutritional approach to limiting inflammation is to use omega-3 fatty acids. These polyunsaturated fatty acids have well-described mechanisms underlying their anti-inflammatory effects, although efficiently delivering them to the small intestine is a challenge in ruminants because of ruminal biohydrogenation of dietary unsaturated fatty acids. Nevertheless, feeding whole flaxseed (omega-3 source) compared to sources of omega-6 fatty acids increased plasma glucose and decreased plasma ketones in fresh cows; more surprisingly, the anti-inflammatory omega-3 source resulted in greater phagocytic activity of circulating leukocytes (Gandra et al., 2016). Although this finding of improved metabolic and immune function is exciting, previous studies have reported indications of less responsive immune systems in cows fed omega-3 sources (Lessard et al., 2003; Silvestre et al., 2011), and such findings are more in line with research in rodents. Perhaps the key to beneficial impacts of omega-3 fatty acids on both inflammation and immunity is an improvement in metabolic profile.

Immune Promotion Tools

With the growing interest in animal characteristics influencing infection risk, a number of factors have emerged as important for supporting strong immunity. Data currently available suggest that cows have improved transition immune function when: 1) they are not exposed to significant heat stress during the dry period (do Amaral et al., 2011); 2) they calve with a BCS between 2.5 and 3.5 (Graugnard et al., 2012; Esposito et al., 2014; Crookenden et al., 2017); 3) they are supplemented with antioxidants during the dry period (Spears and Weiss, 2008); 4) total serum total calcium concentrations are maintained near 9 mg/dL (Martinez et al., 2012; Martinez et al., 2014), and 5) blood BHBA and NEFA concentrations stay below 1 mM during the transition (Grinberg et al., 2008; Contreras and Sordillo, 2011; McArt et al., 2013). Considering the immune system of the transition cow does not necessarily require a change in recommendations for management during this period, but can provide additional motivation to prevent heat stress, provide sufficient access to feed, manage body condition, support calcium homeostasis, and monitor oxidative balance.

Beyond these best practices for transition cow management, a variety of dietary and pharmaceutical products are being marketed for the explicit purpose of improving immune

function. Vaccines have obviously been a very useful tool in promotion of adaptive immunity for decades, and the ongoing development of a vaccine against metritis-causing pathogens may soon bring a new weapon to bear on a frustrating problem (Machado et al., 2014). On the other hand, pharmacological tools for promotion of innate immunity have not been available for livestock until very recently. Granulocyte colony-stimulating factor (GCSF) is a signal used by the immune system which has been adapted into an injectable prophylactic treatment used prior to the period of immunosuppression. The GCSF treatment stimulates the development and maturation of neutrophils, resulting in a fairly dramatic increase in the population of these key innate immune cells in circulation. In conditions favorable to environmental mastitis, the administration of GCSF significantly decreases the incidence of clinical mastitis (Hassfurth et al., 2015).

Dietary agents are also being used as immune modulators, although the exact modes of action for these feed additives are more elusive. We recently reported that a dietary yeast product enhanced antibody response to vaccination and stimulated greater gut release of IgA, which is able to bind to and carry pathogens out of the gut (Yuan et al., 2015). A large-scale analysis of commercial farm responses (off-on) to a different feed supplement was presented recently, suggesting beneficial effects on farm-recorded mastitis and mortality (Chapman et al., 2016). Such dietary components can likely alter the responsiveness of the immune system by interacting with immune sentinels lining the gut and/or by altering gut epithelium function, but other possible mechanisms cannot yet be ruled out.

Is There an Inherent Conflict Between Promoting Immunity and Preventing Excessive Inflammation?

Because inflammation is a core component of the immune system's response to an infection, it is logical to ask whether anti-inflammatory strategies may worsen the immunosuppression that is already recognized as a problem in transition cows. In fact, Nightingale et al. (2015) demonstrated that transition cows with the most dramatic inflammatory profiles also had the most potent measures of neutrophil function. One interpretation of these findings is that transition cows are adapted to respond to immunosuppression with a compensatory inflammatory state.

Inherent conflicts between anti-inflammatory strategies and potent immune responses are also suggested by findings of increased infection rates following NSAID treatments in some small studies and greater mortality rates following pathogen challenges in mice genetically engineered to allow for endogenous omega-3 synthesis (Bradford et al., 2015). Likewise, dietary supplementation of an immune modulator resulted in an increased acute phase response to endotoxin (Brandão et al., 2016), suggesting that at least some means of enhancing immunity will likely promote inflammation as well, although conversely, the febrile response was lessened in this study. In support of the concept that a more appropriate balance between immunity and inflammation can be achieved, the immune stimulant described above resulted in increased milk yield (Brandão et al., 2016), and as mentioned before, post-calving meloxicam treatment increased both milk yield and herd retention (Carpenter et al., 2016).

One question that has not yet been addressed in observational studies is whether the pattern of inflammation impacts long-term outcomes. We hypothesize that brief spikes in inflammatory signals that are resolved in the first 3-4 days of lactation may support immunity and physiological adaptations to lactation. However, failure to rapidly resolve these signals may lead to a variety of adverse impacts that ultimately impair productivity, health, and fertility (Figure 1). We hope that new data will begin to address this question in the coming few years.

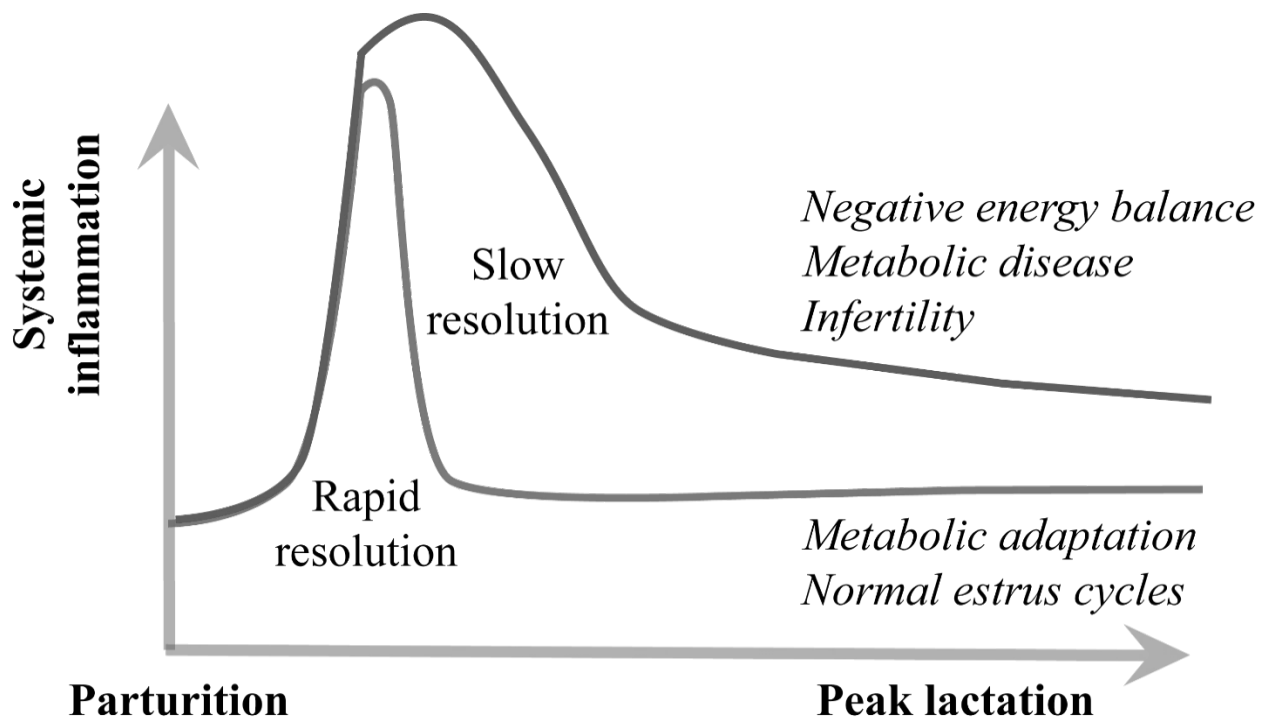


Figure 1. Hypothetical impacts of brief, rapidly resolved postpartum inflammation versus sustained inflammation. It is proposed that lack of resolution leads to impaired health and productivity rather than the inflammation *per se*.

Choosing the Right Strategy for Each Herd

In research with anti-inflammatory agents, there have been some marked differences across studies that, while not allowing strong conclusions, hint at predictors for success with these tools. First, treatment with Banamine shortly before and shortly after calving disrupted the normal process of calving and placental expulsion (Newby et al. 2017), resulting in increased incidence of stillbirths (if given before calving) and metritis (if given after calving). This particular approach to combatting calving-associated pain and inflammation is not advised until at least 24 hours after calving. On the other hand, similar strategies with a different NSAID, meloxicam, did not trigger the same negative effects (Newby et al., 2014).

Second, we have seen variable milk production responses to NSAID treatment even when using identical strategies. Treatment with sodium salicylate for 3 days starting 24 hours after calving increased whole-lactation milk yield by more than 1,000 L in one study (Carpenter et al., 2016), whereas in a follow-up study, we observed no milk response at all (Carpenter et al., 2018). One potentially relevant difference between the cohorts in these two studies is that the responsive group had substantially greater post-calving inflammation, as the mean plasma haptoglobin concentration was more than twice as high in the responsive group compared to the unresponsive group on days 3-4 of lactation. Although we have been unable to demonstrate that individual cows with higher haptoglobin concentrations are more responsive to NSAID treatment, differences between these two studies seem to point in that direction.

Finally, it stands to reason that farms with more infectious disease problems are likely to have more obvious benefits from immune stimulation. As a simple example, on-farm evaluation of responses to a dietary immune modulator showed that decreases in somatic cell count after supplementation began were greatest in herds that started with relatively high somatic cells (Chapman et al., 2016).

Although there is little research basis for this suggestion, mechanisms connecting inflammation and immunity lead to the suggestion that cows in different herds may struggle with different mixtures of transition disorders because of imbalances between pro- and anti-inflammatory signals; excessive inflammation in some herds and inadequate immunity in others. Based on this logic, herds that have relatively high prevalence of infectious diseases in early lactation might be wise to focus on trying immune support tools in an attempt to enhance cows' abilities to combat pathogens. Conversely, herds with more metabolic disorders in early lactation should consider implementing anti-inflammatory management and nutritional strategies. Combinations of both types of supplements may or may not have additive benefits - these interactions simply have not been studied.

Summary

The growing number of tools available to aid cows successfully transition to lactation is exciting, but, as always, the devil is in the details. Several pharmaceutical and feed additive strategies have strong evidence for specific benefits, but individual farms differ in important ways that can lead to unique questions about secondary effects that are less clear. In particular, unresolved

questions about tradeoffs between inflammatory status and immunity make it difficult to give one-size-fits-all recommendations when the transition problems encountered on one farm can differ so dramatically from another. Based on evidence available today, farms with more frequent infectious disease problems are encouraged to explore opportunities to promote immune function, whereas those with prevalent metabolic disorders should perhaps focus more on anti-inflammatory strategies. Research on combinations of such strategies is needed before recommendations can be provided with confidence.

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Risk for Ruminal Acidosis During Finishing

Les risques d'acidose ruminale durant la finition

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S7N 5A8*

Abstract

Finishing beef cattle are fed highly fermentable diets to provide adequate dietary energy and metabolizable protein supply while maximizing the gain-to-feed ratio. These diets increase the rate and extent of fermentation within the rumen and consequently the rate of acid production. Excessive rates of acid production reduce ruminal pH, and increase osmolality and the concentration of microbial associated molecular patterns; all of which may compromise the integrity of the ruminal and intestinal epithelium. Historically, researchers have used low ruminal pH as the single indicator for ruminal acidosis. As a result, numerous reports suggest that feedlot cattle are at high risk for ruminal acidosis during the transition from a backgrounding to finishing diet and throughout the finishing period. However, when evaluating the prevalence, severity, and risk factors for ruminal acidosis in feedlot cattle under commercial settings, we have reported that under well managed conditions, the prevalence is high but severity for ruminal acidosis is low during the diet transition period in group fed cattle. That said, our data suggest that the terminal phase of the finishing period may be a time with greater risk potentially due to environmental conditions or the sensitivity of cattle themselves to ruminal acidosis as they experience repeated bouts of excessive ruminal fermentation and increased body fat content. Overall, the severity of ruminal acidosis during finishing is quite low under well-managed conditions. While ruminal pH is relatively easy to measure, conditions related to ruminal acidosis that lead to the secondary disorders observed in finishing cattle (ruminitis, liver abscesses, and laminitis) have eluded researchers. We recently reported that cattle with ruminal and/or liver pathology at slaughter had greater concentrations of circulating acute phase proteins and spent more time with ruminal pH < 5.2 during the finishing period. Thus, our data suggest that a combination of severely depressed low pH and increased antigen concentration may help predict cattle at risk for secondary disorders arising from ruminal acidosis.

Résumé

Les bovins de boucherie à l'engraissement reçoivent des rations composées d'ingrédients très fermentescibles afin de fournir suffisamment d'énergie et de protéines métabolisables et de maximiser l'indice de consommation alimentaire. De telles rations favorisent une fermentation plus rapide et complète dans le rumen, ce qui entraîne une hausse du taux de production d'acides. Des taux excessifs de production d'acides abaissent le pH ruminal et augmentent l'osmolalité et la concentration de motifs moléculaires associés aux microorganismes, autant de phénomènes susceptibles de compromettre l'intégrité des épithéliums du rumen et des intestins. Depuis toujours, le seul indicateur de l'acidose ruminale utilisé par les chercheurs est le faible pH

ruminal. Par conséquent, de nombreuses études suggèrent que les bovins en parc d'engraissement sont grandement menacés par l'acidose ruminale pendant la période de transition de la ration de semi-finition à la ration de finition et pendant l'ensemble de la période de finition. Cependant, dans notre évaluation de la prévalence, de la sévérité et des facteurs de risque de l'acidose ruminale chez les bovins en parc d'engraissement dans un contexte commercial, nous avons observé que, dans de bonnes conditions de régie, la prévalence de l'acidose ruminale est certainement élevée, mais que ce trouble est peu sévère pendant la transition chez les bovins alimentés en groupe. Par contre, nos données montrent que la dernière phase de la finition pourrait présenter davantage de risques, probablement en raison des conditions environnementales ou de la susceptibilité des bovins à l'acidose ruminale, au moment même où ils doivent composer avec des épisodes répétés de fermentation ruminale excessive et un taux de graisse corporelle accru. Globalement, la sévérité de l'acidose ruminale durant la phase de finition est plutôt faible, quand les conditions de régie sont acceptables. Bien que le pH du rumen soit relativement facile à mesurer, les conditions associées à l'acidose ruminale à l'origine des désordres secondaires observés chez les bovins en finition (ruminite, abcès du foie et fourbure) échappent encore aux chercheurs. Nous avons récemment rapporté que les bovins atteints d'une pathologie du rumen ou du foie à l'abattage avaient présenté de plus fortes concentrations de protéines de phase aiguë en circulation et avaient passé davantage de temps à pH ruminal $< 5,2$ durant la période de finition. Ainsi, nos données suggèrent que l'observation de valeurs de pH largement inférieures à la normale jumelées à une hausse de la concentration d'antigènes pourrait aider à identifier les bovins à risques de désordres secondaires consécutifs à l'acidose ruminale.

Introduction

Feedlot cattle are often fed highly fermentable diets to achieve rapid rates of gain and minimize the quantity of carcass gain per unit of feed (**G:F**). While feeding highly fermentable diets generally improves growth and G:F, excessive rates of acid production in the rumen can overwhelm the ability of cattle to regulate ruminal pH and consequently may increase the risk and severity of ruminal acidosis. Therefore, with highly fermentable diets, it is not surprising that ruminal acidosis is often cited as the most common digestive disorder affecting feedlot cattle (Owens et al. 1998).

Ruminal acidosis has been suggested to reduce individual animal performance and is a predisposing factor for secondary disorders such as liver abscesses and lameness (Nocek 1997; Owens et al. 1998; Nagaraja and Titgemeyer, 2007). A recent study revealed that ruminal acidosis (defined to occur when $\text{pH} < 5.5$) is negatively related to G:F and average daily gain (**ADG**; Castillo-Lopez et al. 2014). Thus, management strategies to mitigate ruminal acidosis may yield positive effects on growth performance and G:F in addition to improvements in animal welfare. However, prior to implementing management strategies, it is important to understand the risk and severity of ruminal acidosis during finishing to determine when intervention strategies may be most needed and provide the greatest benefit.

Definition and Outcomes of Ruminal Acidosis in Feedlot Cattle

The definition of ruminal acidosis has been challenged and has evolved over the last 15 yr. Ruminal pH has been used to define thresholds where reducing pH further induces negative effects. Such negative effects, and therefore thresholds, have focused on activity of the microbial population and cattle themselves. Originally, sub-acute ruminal acidosis was defined to occur when ruminal pH was below 5.8 and acute ruminal acidosis when pH was below 5.0 (Owens et al. 1998). It is clear that exposure to low pH (< 6.0) reduces the digestibility of neutral detergent fibre (**NDF**), but the pattern of low pH depression (number of bouts and duration of bouts) influences the magnitude of this response (Calsamiglia et al., 2002). For feedlot cattle fed diets common in western Canada, the contribution of dietary energy supply from NDF is low (Joy et al., 2016) and hence a ruminal pH threshold focusing on NDF digestibility is likely not rational.

Focusing on the impact for cattle, low ruminal pH itself does not always present a problem to cattle and there have been suggestions to include additional criteria (Khafipour et al. 2009) into the definition for ruminal acidosis. The additional factors focus on the potential for low ruminal pH to increase the antigen concentration in the rumen (Gozho et al. 2005, 2006), compromise the ruminal epithelial barrier function (Gaebel et al., 1987), and lead to increased concentration for indicators of a system immune response (i.e. acute phase proteins; Gohzo et al., 2006; Khafipour et al., 2009). Using a challenge where the forage-to-concentrate ratio was rapidly decreased, Gozho et al. (2005) demonstrated that the concentration of lipopolysaccharide (**LPS**) in ruminal fluid increased with increasing concentration of grain in the diet and that such responses also led to a reduction in ruminal pH and an increase in acute phase proteins in blood. They further suggested that the increase in acute phase protein concentrations did not occur until pH was below 5.6 for more than 3 h/d. This suggests that both the pH threshold and the duration that pH is maintained below that threshold need to be considered.

In feedlot cattle, ruminal acidosis is often associated with a marked increase in ruminal fluid lactate concentration (Owens et al. 1998; Brown et al. 2000). Lactate is slowly absorbed across the rumen wall (Harmon et al. 1985) and is a stronger acid than most abundant short-chain fatty acids (**SCFA**). Thus, lactate can reduce ruminal pH lower than SCFA and can sustain a reduction in ruminal pH for a greater duration. Although increased concentration of lactic acid in ruminal fluid increases lactic acid absorption from the rumen, rarely does ruminal acidosis result in metabolic acidosis (Harmon et al. 1985).

Recently the impact of ruminal acidosis on other regions of the gastrointestinal tract has been highlighted. For example, Pederzoli et al. (2018) reported that induction of ruminal acidosis decreased the pH in the rumen, but also decreased pH in the cecum and throughout the colon relative to cattle where acidosis was not induced (Figure 1). They also noted changes in gene expression for proteins related to barrier function of the tissue supporting the suggestion that ruminal acidosis may have a much broader effect than events isolated to the rumen. Plaizier et al. (2014) reported that LPS concentrations also vary across the gastrointestinal tract and pH-induced alterations to barrier function in the intestinal tract may serve as a location where inflammation is initiated and for pathogen translocation. However, currently there is no data to conclusively determine which region or regions of the gastrointestinal tract and the most affected regions by ruminal acidosis.

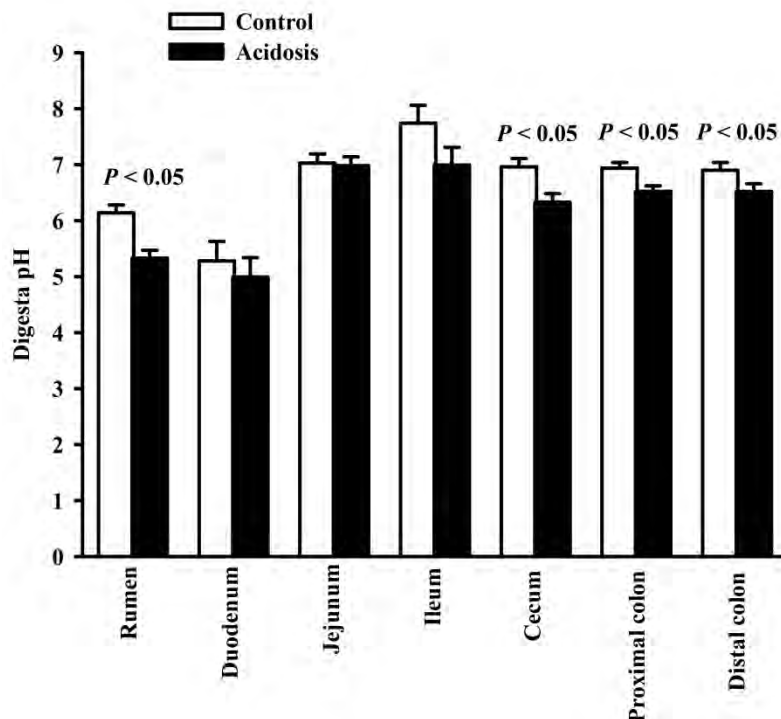


Figure 1. The effect of inducing ruminal acidosis, using 1 d of feed restriction coupled with overfeeding the following day, on digesta pH across the gastrointestinal tract. Adapted from Pederzoli et al. (2018).

The relationship between ruminal acidosis and cattle growth performance is complex and multifactorial. Across studies, increasing starch intake generally increases ADG and G:F, and over entire experiments may indicate that low ruminal pH improves pen-based measures of performance for cattle. However, there may be times during the feeding period when this relationship is inverted: ruminal acidosis may reduce ADG and G:F. Castillo-Lopez et al. (2014) investigated ruminal pH of cattle during the backgrounding, diet transition and finishing phases using ruminally cannulated steers with indwelling pH probes. The backgrounding (d 1 to 20; 45.7% barley silage, 41.6% barley grain), diet transition (d 21 to 40; 4 step-ups), and the first (d 41 to 91; 5% barley silage, 80.9% barley grain) and second half (d 92 to 143) of finishing were evaluated independently. Prevalence of ruminal acidosis (defined as the percentage of steers with ruminal pH < 5.5 for > 3 h/d) increased across the feeding period (0.7, 1.7, 15.4 and 37.8 ± 2.0%, respectively; Figure 2). The duration that pH was less than 5.5 was strongly positively correlated ($P < 0.001$) with DMI, and negatively correlated with ADG and G:F indicating that cattle with reduced pH had reduced ADG and G:F. Although incidence of acidosis relatively low in this study, it clearly illustrates negative relationships between growth performance and feed efficiency throughout the feeding period. A study (Rezac et al., 2014) evaluating the prevalence and severity of lung, liver and rumen lesions and their associated relationships in over 13,000 slaughtered beef cattle from Kansas and Texas also supports this negative relationship. Of the observed cattle 24.1% were found to have ruminitis lesions and scars, indicative of a bout of

severe ruminal acidosis. The incidence of severe rumen lesions was associated with decreased ADG ($P < 0.001$) and 2.2 kg depression ($P < 0.01$) in carcass weight, independent of the influence of liver abscesses. Supporting this finding, Wiese et al. (2017a) evaluated the relationship between ruminal pH and evidence of ruminal acidosis driven pathological changes including ruminal abscesses and liver abscess. In that study, they found that steers with ruminal and liver pathology had greater time with ruminal pH below 5.2 and had increased concentrations of acute phase proteins. From a clinical perspective, it may appear that lower pH values than previously suggested may be necessary to reflect outcomes affecting health and performance of feedlot cattle.

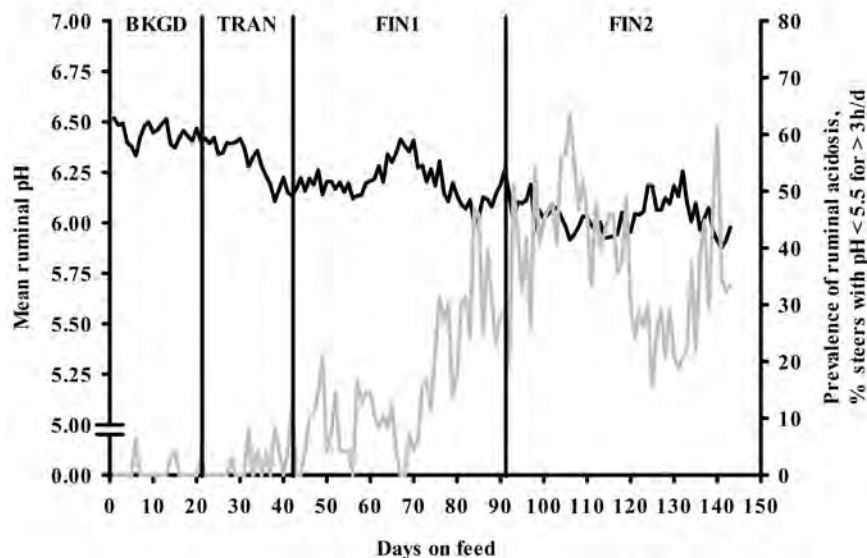


Figure 2. Mean ruminal pH and prevalence of ruminal acidosis during backgrounding (BKGD; d 1 to 20), dietary transition to a finishing diet (TRAN; d 21-41), and finishing (FIN; d 42 to 141). Figure from Castillo-Lopez et al. (2014).

The broad spanning impact of ruminal acidosis along with ambiguous and transient symptoms (variable feed intake, and in clinical cases diarrhea and depression; Owens et al. 1998) present a challenge in developing rapid diagnostic tools that can be used in the field. Thus, ruminal pH remains as the most appropriate sole indicator of ruminal acidosis with blood parameters such as acute phase proteins enhancing the diagnosis under research conditions. It is important to emphasize that individual researchers utilize different pH thresholds and criteria to define ruminal acidosis, thus the definition of ruminal acidosis is variable across studies, which affects the prevalence of acidosis reported.

Understanding Critical Risk Periods for Ruminal Acidosis

Many nutritionists and producers would agree that the dietary transition from a primarily forage-based diet to a high-grain finishing diet is a period of high risk for ruminal acidosis. Currently,

the adaptation system most commonly used is a step-up program (Vasconcelos and Galyean 2007; Millen et al. 2009), in which three or four rations are utilized depending on the level of energy contained in the finishing diet.

Numerous studies have been conducted to evaluate dietary adaptation strategies and risk factors for ruminal acidosis during the dietary transition period (Choat et al. 2002). One study using beef heifers investigated a rapid adaptation to a 90% grain diet (65% for 3 d) vs. a gradual adaptation (five step-up diets fed 3 d each) and indicated that no differences for mean ruminal pH were observed, although variation in pH was greater for the rapid adaptation protocol potentially indicating greater risk for ruminal acidosis (Bevans et al. 2005). Results from these studies emphasize that a gradual dietary transition helps to minimize the substantial variation in ruminal pH response among individual animals within a group. A recent study investigated ruminal pH and feeding behavior over 4 d of each step-up period in cannulated beef heifers adapted to a 90% concentrate diet over a three wk period, with three step-up diets (Holtshausen et al. 2013). Mean ruminal pH, DM intake (DMI), meal size and duration decreased within each step-up period and DMI and meal size decreased across the step-up period with the severity of acidosis increasing as the level of concentrate in the diet increased. Although the duration of adaptation is only a small portion of the total feeding period, adapting cattle too rapidly can have adverse long-term consequences and adapting cattle too slowly can increase the cost of weight gain (Brown et al., 2006).

Despite the focus on the dietary transition period as a critical risk period, a recent study has indicated that the prevalence rates for ruminal acidosis during the dietary transition period range between 0 and 9.1% (Figure 2; Castillo-Lopez et al. 2014). This study utilized a definition for ruminal acidosis of ruminal pH < 5.5 for more than 3 h/d and utilized a 20-d dietary transition protocol with 4 diets until achieving a final diet containing (inclusion, % DM basis) barley silage (5), barley grain (81), canola meal (5), mineral and vitamin supplement (8), and limestone (1). A second study (Wiese et al., 2017b) conducted in a commercial feedlot using orally dosed ruminal-reticulo pH measurement also demonstrated high prevalence rates but low severity of ruminal acidosis in group-fed cattle in a commercial setting. Collectively, these data suggest that current management practices have been effective at mitigating the severity of ruminal acidosis during the dietary transition.

Environmental conditions are rarely considered as a risk factor for ruminal acidosis. In the study of Castillo-Lopez et al. (2014) prevalence rates during the entire finishing phase ranged between 0 and 60%. The points in time with greatest risk for ruminal acidosis anecdotally corresponded with increasing environmental temperatures that may impact the depth of mud in the pen conditions. To address this issue, a study was conducted to evaluate the effect of pen conditions on ruminal acidosis. Using 8 pens containing nearly 300 steers/pen, Castillo-Lopez et al. (unpublished) measured reticulo-ruminal pH as mud depth changed. Pens were retrospectively classified based on the increase in mud depth allowing for 4 pens with no increase in mud depth (1 cm increase) or an average of a 6 cm increase in mud depth for the deep mud pens. The pens with the greater mud depth had a fewer meals during a 12-h observation period than those with less mud but had longer meal times without differences for total DMI. Although not measured, this would imply that the quantity of feed consumed at each meal increased which may increase risk for ruminal acidosis. Despite low risk for reticulo-ruminal acidosis in that study, the increase

in the duration of time spent below 5.5 was greater for pens with an increase in mud depth. Prevalence rates tended to be greater for the deep mud pens than the shallow mud pens with prevalence rates of 17 vs. 9%. Again, although the severity of low ruminal pH was low in that study, the potential increase in association with mud depth highlights the importance of pen conditions in affecting feeding behavior.

Data from Castillo-Lopez et al. (2014) suggest that cattle in the finishing phase, and likely the latter portion of the finishing phase may be most susceptible to ruminal acidosis. This may challenge conventional thinking as it is commonly suggested that adaptation to a highly fermentable diet should reduce the risk for ruminal acidosis. However, results from Dohme et al. (2008) suggest that induction of ruminal acidosis increases the susceptibility of cattle to subsequent bouts of ruminal acidosis. To clarify whether adaptation reduces risk, we evaluated whether the duration of time cattle were fed a high-grain diet (81% barley grain, 10% mineral and vitamin supplement, and 9% barley silage on DM basis) affected the risk for ruminal acidosis (Schwaiger et al. 2013a,b). In that study, cattle were adapted to the final diet over 20 d using 5 dietary steps and then were fed the final diet for 8 d for the short-adapted or for 34 d for the long-adapted treatment before inducing ruminal acidosis. The results of that study demonstrated that the duration of time that cattle were fed the high-grain diet prior to inducing ruminal acidosis did not affect the risk of ruminal acidosis; however, the ruminal pH for long-adapted cattle recovered more rapidly. These data were interpreted to indicate that the risk for ruminal acidosis is similar for beef cattle regardless of the number of days they are fed a high-grain diet, but cattle fed a high grain diet for a greater duration may be better equipped to respond and recovery more rapidly. Future research is needed to evaluate how repeated episodes of ruminal acidosis may affect this response and to evaluate production outcomes.

Conclusions

Ruminal acidosis is a prevalent digestive disorder in feedlot cattle but the severity of the episodes are mild. The diet transition period appears to impose high risk but low severity of ruminal acidosis while later in finishing both the risk and severity increase. As such, it is clear that high DMI and long days on feed coupled with highly fermentable rations increase the risk.

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Fetal Programming and Its Implications for the Beef Industry

La programmation fœtale et ses répercussions pour l'industrie du bœuf

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Abstract

Nutrient deficiency often occurs in animals provided forage-based diets due to seasonal variation in forage quality and quantity. This nutrient deficiency has been shown to impact reproductive performance of cows, but also fetal development. Maximal placental growth, differentiation, and vascularization occur during the early phase of fetal development, whereas muscle and adipose tissue development occurs primarily from mid-gestation to calving. Thus, calf development during gestation is susceptible to maternal nutrition, which might lead to long-term effects on postnatal growth and reproductive performance of calves. Protein supplementation during the last trimester of gestation improved weaning weight of steers and heifers, post-weaning health of steers, carcass quality of steers, and puberty attainment and pregnancy rates of beef heifers compared to offspring born to non-supplemented cows. Hence, closer attention and proper nutrition of the herd need to be enforced to avoid or alleviate the negative impacts of nutrient restriction during gestation on cow and calf performance. Research from University of Florida is currently focusing on the use of year-round supplementation or late-gestation supplementation of energy and protein to modify calf development during gestation and improve postnatal health, growth, and reproductive performance. Both strategies significantly improved growth performance and physiological parameters of pregnant beef cows. Calf performance and health following birth are still under investigation.

Résumé

Il n'est pas rare que les animaux qui reçoivent des rations à base de fourrages souffrent d'une carence en nutriments en raison des variations saisonnières dans la qualité et la quantité des fourrages. Une telle carence nutritionnelle s'est avérée nuisible pour la performance de reproduction des vaches, mais aussi pour le développement fœtal. Le pic de croissance, de différenciation et de vascularisation du fœtus survient au début de la gestation, tandis que les tissus musculaires et adipeux se développent surtout entre le milieu de la gestation et le vêlage. Ainsi, le développement du veau durant la gestation repose sur l'alimentation de la mère, ce qui peut entraîner des effets à long terme sur la croissance après la naissance et sur la performance de reproduction des jeunes animaux. La supplémentation protéique au cours du dernier trimestre de la gestation a amélioré le poids au sevrage des bouvillons et des génisses, la santé des bouvillons post-sevrage, la qualité de carcasse des bouvillons ainsi que le rythme d'atteinte de la

puberté et le taux de gestation des génisses de boucherie, comparativement aux nouveau-nés de vaches qui n'ont pas reçu le supplément. Il est donc important de porter davantage attention au troupeau ainsi qu'à sa nutrition afin d'éviter ou du moins d'atténuer les effets négatifs des restrictions nutritionnelles durant la gestation sur la performance des vaches et des veaux. Des travaux en cours à University of Florida portent sur l'utilisation durant toute l'année ou en fin de gestation de suppléments énergétiques et protéiques dans le but de modifier le développement du fœtus et d'améliorer la santé, la croissance et la performance de reproduction après sa naissance. Les deux stratégies ont amélioré significativement la performance de croissance et les paramètres physiologiques des vaches de boucherie gestantes. La performance des veaux et la santé après la naissance sont toujours à l'étude.

Introduction

The beef cattle industry relies primarily on the use of high-forage diets to develop replacement heifers and maintain cow herd and stocker operations. However, forage quantity and quality changes with season and environmental conditions. Depending on the physiological state and animal category, forage-based diets may not always meet 100% of the nutrient requirements, resulting in body weight loss or reduced performance if supplemental nutrients are not provided (Funston et al., 2012). Cattle experience nutrient restriction more often than realized because of overgrazing and lack of forage frequently observed throughout the country.

It is well reported in the literature that cows will not conceive at an acceptable rate without having adequate body fat reserves at calving (BCS = 5; 1 to 9 scale). A low BCS at the time of calving (less than 5) extends the *anestrous period*, which is the period when the cow is recovering from calving and is not cycling. An extended anestrous period decreases the percentage of cows that are cycling and able to breed at the start of the breeding season, leading to lower pregnancy rates. In addition, pregnancy will probably occur at the end of the breeding season, delaying the subsequent calving and leaving less time to recover before the next breeding season. Recently, multiple studies have demonstrated that cow nutrition can impact more than just pregnancy rates. In this publication, we will summarize some of the recent data showing the effects of poor nutrition of *Bos taurus* cows on subsequent calf growth and health (Fetal programming), and the discuss the current research studies being conducted by our laboratory at the Range Cattle Research & Education Center - University of Florida.

Fetal Programming

Fetal programming is the concept that a maternal stimulus or insult at a critical period in fetal development has long-term effects to the offspring (Funston et al., 2010). Approximately 75% of calf fetal growth occurs during the last two months of gestation (Robinson et al., 1977). Calf nutrient requirements are therefore relatively low during the first two trimesters of gestation. For that reason, many people believed that cow nutrition could only affect calf growth during the last trimester of gestation. Recent data demonstrate that this is not the case.

Maximal placental growth, differentiation, and vascularization occur during the early phase of fetal development. The placenta is the major regulator of calf fetal growth, and it appears that maternal nutrition may affect the development and function of the placenta (Funston et al., 2010). In addition, most organs form simultaneously with placental development during early gestation. For instance, pancreas, liver, adrenals, lungs, thyroid, spleen, brain, thymus, and kidneys start to develop at 25 days of pregnancy (Hubbert et al., 1972). Each organ and tissue has its own “window” of formation. For example, organs such as kidneys and pancreas occur during early gestation, whereas muscle and adipose tissue formation occurs primarily during mid- to late-gestation (Du et al., 2010). Thus, nutrient restriction or excess during gestation might impact placental formation and calf organ development. Also, depending on when the nutrient restriction or excess occurs during gestation, the outcome of this insult to calf performance might differ

Muscle and Adipose Tissue Formation

Muscle fiber development can be separated into prenatal and postnatal stages (Du et al., 2017). During bovine fetal development, the formation of primary muscle fibers occurs within the first 2 months after conception (Russell and Oteruelo, 1981), whereas the formation of secondary muscle fibers (which represents most of muscle fibers) occurs between 2 and 7 months of gestation (Du et al., 2010). Subsequently, the formation of new muscle fibers is limited, and muscle growth occurs primarily by the increase in size and length of each fiber (Figure 2; Du et al., 2017). Therefore, reducing the proliferation of precursors for muscle fibers decreases the number of fibers, which has irreversible negative effects on offspring muscle growth after birth (Zhu et al., 2006). Proper maternal nutrition provides nutrients needed for proliferation and formation of muscle fibers (Du et al., 2017). Because skeletal muscle has a lower priority in nutrient partitioning compared with the brain, heart, and liver, muscle development is highly vulnerable to the variation in nutrient availability (Zhu et al., 2006). Also, insufficient maternal nutrition reduces the concentration of insulin growth factors 1 and 2 (IGF-1 and IGF-2) in fetal circulation, which also decreases the development of muscle fibers (Gonzalez et al., 2013).

Adipose tissue development is also profoundly affected by maternal nutrition. There are 4 fat tissue depots in animals: visceral, subcutaneous, intermuscular, and intramuscular fat (Du et al., 2017). Intramuscular fat (also referred as marbling) contributes to the flavor and juiciness of meat. However, visceral, subcutaneous, and intermuscular fat comprise the majority of body fat tissue reserves and have low commercial value. To accumulate these fat tissue reserves, a large amount of nutrients is required, leading to a reduction on feed efficiency (Du et al., 2017). Therefore, studies are being conducted to enhance marbling deposition while reducing fat accumulation in other depots. The mass of fat tissue is determined by the number and size of adipocytes. The number of adipocytes is mostly determined during the fetal and early-postnatal development, reaching maximum total number of adipocytes at adolescence (Spalding et al., 2008). In beef cattle, visceral and subcutaneous fat develop primarily from mid-gestation to neonatal stage, whereas the formation of intramuscular adipocytes occurs primarily from late gestation until approximately 250 days of age (Figure 1). This chronological difference in adipocyte formation provides an opportunity to enhance marbling adipocyte formation without increasing overall adiposity of beef cattle (Corah and McCully, 2007; Du et al., 2013).

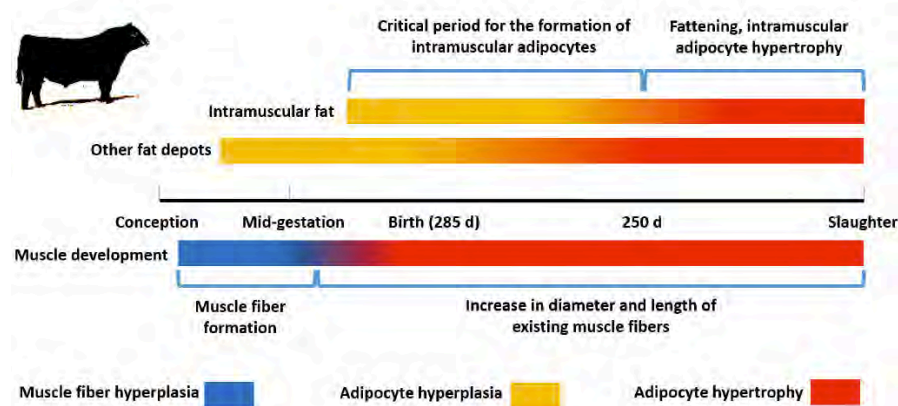


Figure 1. Timeline of muscle and adipose tissue development in beef cattle (Du et al., 2017).

Consequences of Nutrient Restriction to *Bos Taurus* Cattle

Early Gestation (0 to 3 Months of Gestation)

Cows must conceive within 80 days postpartum if a yearly calving interval is desired. Cows' milk production and nutrient requirements peak at 60 days postpartum; however, intake lags behind. This results in negative energy balance during early to mid-lactation (NRC, 2016), especially if calving occurs during winter seasons when poor forage quality and quantity is available. Unfortunately, few studies evaluated the effects of cow nutrient restriction during early gestation on beef calf performance. A University of Wyoming study evaluated the growth performance and organ development of calves born to cows experiencing nutrient restriction during first trimester of gestation (Long et al., 2010). In that study, cows were separated into two groups that were fed at 55 or 100% of their nutrient requirements for the first 83 days of gestation. Following 83 days, both groups were provided 100% of their nutrient requirements until calving. Logically, cows provided 55% of their nutrient requirements lost 137 lb of body weight, whereas cows fed 100% of their nutrient requirements gained 95 lb of body weight during the first 83 days of gestation. No differences were observed for calf birth weight, weaning weights, and average daily gain from birth to weaning or during the feedlot finishing phase. However, lung and trachea weights of steers born to cows provided 55% of their nutrient requirements were significantly less than those from steers born to cows fed 100% of their nutrient requirements (Figure 2). Although growth performance was not affected, it would be misleading to interpret these results as if nutrient restriction during early gestation could not impact calf performance. In a commercial feedlot, calves are constantly exposed to several pathogens and commingled with calves of unknown health background. It is therefore possible that smaller lungs could be detrimental to calf performance if those calves experience bovine respiratory disease after entering a commercial feedlot. However, additional studies are needed to confirm this hypothesis.

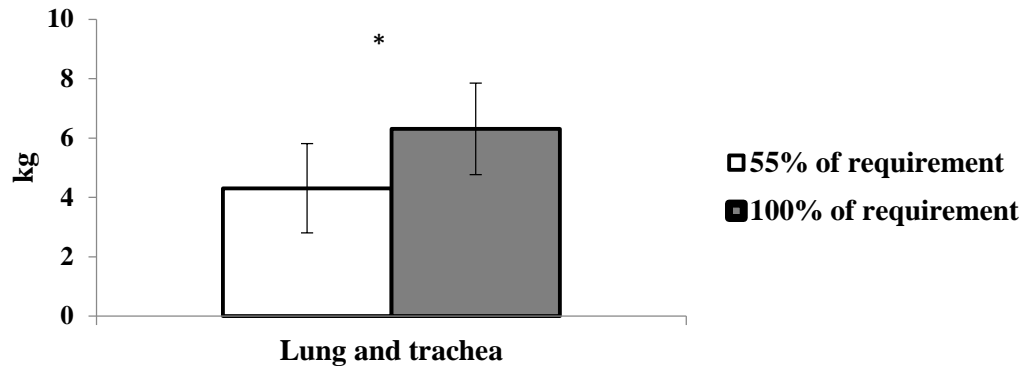


Figure 2. Lung plus trachea weights of steers born to first-calf heifers provided 55 or 100% of their nutrient requirements during the first 83 days of gestation. * $P < 0.05$

Mid Gestation (3 to 6 Months of Gestation)

As described above, nutrient restriction during mid gestation is expected to decrease muscle fiber formation, leading to lower birth and weaning weights. At the University of Wyoming, researchers evaluated the growth performance of steers born to cows that grazed low-quality, native pastures (6% crude protein) or high-quality, fertilized and irrigated pastures (11% crude protein) from 120-150 days of gestation until 180-210 days of gestation (Underwood et al., 2010). In that study, researchers reported that body weight at weaning and carcass weights were reduced for male offspring born to cows that grazed native pastures compared to male offspring born to cows that grazed improved pastures during mid-gestation (Table 1). In addition, the Warner-Bratzler shear force (indicator of meat tenderness) was less for *Longissimus* muscle samples of male offspring born to cows grazed on improved pastures (31 vs. 37 N; $P = 0.004$). In summary, cows that grazed on improved pastures during mid-gestation produced calves that were heavier at weaning and harvesting, and that had greater meat tenderness at slaughter.

Nutrient restriction during mid-gestation may also have consequences to organ development. Angus × Gelbvieh cows were fed 70% or 100% of their nutrient requirements from day 45 to 185 of gestation. Thereafter, cows were commingled and fed to meet 100% of their nutrient requirements from day 185 of gestation until calving (Long et al., 2012). Although body weight at birth and at weaning did not differ ($P \geq 0.19$) between treatments, heifers born to cows fed at 70% of their nutrient requirements had smaller ovaries and luteal tissue (Figure 3). Luteal tissue is crucial for progesterone synthesis and pregnancy maintenance. Therefore, smaller ovary and luteal tissue could affect cows' reproductive performance during their first breeding season. Additional studies are required in this area to confirm these results and evaluate long-term effects of nutrient restriction during mid gestation on subsequent reproductive performance of the heifer progeny.

Table 1. Growth performance of male offspring born to cows grazed on native (6% crude protein) or improved pastures (11% crude protein) for 60 days during mid gestation (Underwood et al., 2010).

	Grazing management during mid gestation		SEM	P-value
	Native pastures	Improved pastures		
Birth, kg	39	36	2.0	0.46
At weaning, kg	242	256	3.7	0.02
At slaughter, kg	520	544	7.7	0.04
Hot carcass weight, kg	330	348	4.8	0.04

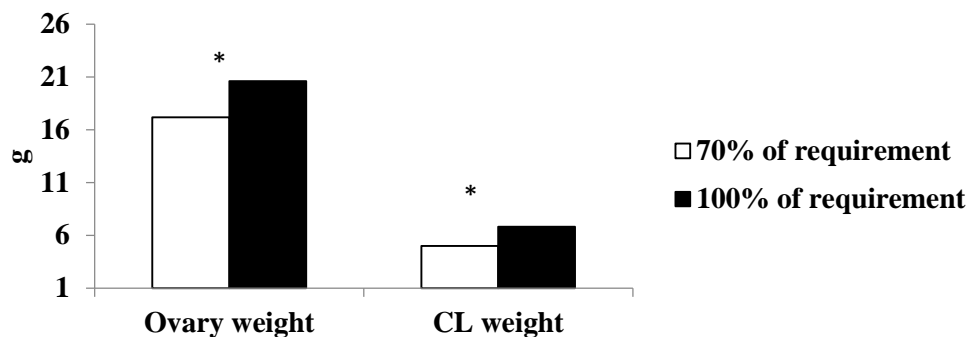


Figure 3. Wet ovary and luteal tissue weights of heifers born to cows provided 70 or 100% of their nutrient requirements from 45 to 185 days of gestation (Long et al., 2012). * $P < 0.05$

Late Gestation (6 to 9 Months of Gestation)

Late gestation is probably the most important gestation period in terms of potential impact on production-oriented tissues such as muscle and adipose tissue. The final number of muscle fibers is set at birth, meaning that after birth, there is no net increase in the number of existing muscle fibers. Thus, if nutrient restriction during late gestation reduces muscle fiber number (Zhu et al., 2004), calf growth performance following birth might be compromised. In addition, maternal nutrient restriction may also compromise adipocyte populations (cells responsible for intramuscular fat, for example), resulting in carcasses with lower quality and marbling scores.

In a series of studies (Stalker et al., 2006, 2007; Larson et al., 2009), researchers evaluated the effects of providing protein supplementation during late gestation on subsequent offspring performance (Table 2). Cows were sorted into groups that received or did not receive 0.454 kg/day of a protein supplement (42% crude protein) for 90 days before calving. All studies reported that the male offspring born to cows that received protein supplementation were heavier than male offspring born to non-supplemented cows. Also, two of those studies (Stalker et al., 2007; Larson et al., 2009) reported heavier carcasses for males born to cows that were supplemented with protein, whereas one study (Larson et al., 2009) reported greater percentages

of carcasses grading Choice and greater marbling scores for steers derived from cows that received supplementation.

Similar studies evaluated the performance of heifer offspring born to cows supplemented with 0.454 kg/day of a protein supplement during late gestation. In those studies, weaning weights (Martin et al., 2007) and weights adjusted for 205 days of age (Funston et al., 2010) were greater for heifers born to cows that received protein supplementation. Also, heifers born to cows that were supplemented achieved puberty sooner (Funston et al., 2010) and had greater pregnancy rates (Martin et al., 2007) than heifers born to cows that did not receive supplementation (Table 3).

Table 2. Performance of steers born to cows that received (Supp.) or did not receive (No Supp.) protein supplementation (0.454 kg daily of a 42% crude protein supplement) * $P < 0.05$

Item	Stalker et al. (2007)		Stalker et al. (2006)		Larson et al. (2009)	
	No Supp.	Supp.	No Supp.	Supp.	No Supp.	Supp.
Weaning weight, kg	200*	210*	211*	218*	235*	241*
Carcass weight, kg	347*	365*	363	369	364*	367*
Choice, %	-	-	85	96	71*	86*
Marbling scores	449	461	467	479	444*	493*

Table 3. Performance of heifers born to cows that received (Supp.) or did not receive (No Supp.) protein supplementation (0.454 kg daily of a 42% crude protein supplement). * $P < 0.05$

Item	Martin et al. (2007)		Funston et al. (2010)	
	No Supp.	Supp.	No Supp.	Supp.
Weaning weight, kg	207	212	225*	232*
Adj. 205-day weight, kg	218*	226*	213	217
Age at puberty, days	334	339	366*	352*
Pregnancy rate, %	80*	93*	80	90

Offspring Health

Few reports have focused on the effects of maternal nutrition during gestation on calf health. Corah et al. (1975) reported increased morbidity and mortality rates in beef calves born to primiparous cows receiving 65% of their dietary energy requirement over the last 90 days of gestation compared to calves from primiparous cows receiving 100% of their energy requirement. A potential factor contributing to an increased morbidity and mortality is the decreased calf birth weight. Calves born from nutrient-restricted cows were 2.3 kg lighter at birth compared to calves born from cows receiving adequate nutrition (Corah et al., 1975). Larson et al. (2009) observed no differences in the number of calves treated for bovine respiratory disease (BRD) from birth to weaning. However, less calves had to be treated for BRD after feedlot entry if they were born from cows provided 0.454 kg/day of a protein supplement for the last 90 days of gestation compared to calves from non-supplemented cows. Stalker et al. (2006) reported increased proportions of live calves weaned to dams offered supplement during late gestation;

however, there was no difference in the number of calves treated for BRD before weaning or in the feedlot.

Our research conducted at North Carolina State University reported no differences on calf birth weight and pre-weaning growth performance of calves born from cows that received either 70% or 100% of their energy requirements during the last 40 days of gestation (Moriel et al., 2016). However, calves born from cows that were fed 70% of energy requirements had lower overall plasma concentrations of cortisol (indicator of stress level) and haptoglobin (indicator of inflammatory response) compared to calves born from cows fed at maintenance levels (Table 4). Also, calves born from cows that were energy-restricted during late gestation produced less antibodies against bovine viral diarrhea virus (BVDV), which is one of the main pathogens that cause BRD. These results together indicate that calves born to cows that were energy-restricted for just 40 days before calving had an immune system that is not responsive than calves born from cows that were fed at maintenance levels. Therefore, even though calf growth performance was not affected, calves might be more susceptible to diseases if they are born from cows that were energy-restricted. More studies need to be conducted in this area as it has substantial implications to cow-calf producers, and this need will be addressed by our research group at Ona, FL.

Table 4. Immune response of calves born to beef cows offered diets formulated to meet 100% of energy requirements (Maintenance) or 70% of energy requirements (Restricted) during late gestation (day 0 until calving; approximately 40 days before calving; Moriel et al., 2016).

	Maternal Diet			
Item	Maintenance	Restricted	SEM	P-value
<i>Post-weaning phase</i>				
ADG (42 days after weaning), kg/day	0.82	0.86	0.059	0.59
Plasma cortisol, ng/mL	17.5	13.7	1.53	0.05
Plasma haptoglobin, mg/mL	0.53	0.42	0.043	0.10
<i>Serum antibody titers against BVDV-1a, log₂</i>	6.36	5.15	0.463	0.05

Fetal-programming Research in Florida Beef Herds

STUDY #1: Does Year-round Supplementation of Cows Pay Off?

Most of FL cow-calf operations provide year-round supplementation of trace minerals, but provide protein and energy supplementation to alleviate cow weight loss only during early-lactation. However, inadequate dietary energy/protein during late pregnancy lowers reproduction even if the amount of energy and protein consumed during early-lactation are sufficient (Perry et al., 1991). Recent studies have shown that nutritional insults during gestation can also modify placental development, fetal organ formation, and offspring growth and health. Thus, the decisions about cowherd supplementation should also include the impact on future offspring performance. One strategy that might improve cow reproductive success and offspring

performance following birth is the use of year-round supplementation. In this scenario, cows supplemented year-round might achieve a greater body condition score at calving (November) without increasing the annual supplement amount. Another advantage is that the trace mineral salt will be mixed into the supplement, reducing annual fluctuations in voluntary intake and wastage of free choice trace mineral formulations, and improving cow trace mineral status. Our hypothesis is that year-round supplementation of molasses or range cubes (isocaloric and isonitrogenous supplements) will increase body condition score at calving and trace mineral status of cows throughout the year, which will enable cows to experience greater body condition loss during early-lactation without reducing their reproductive performance compared to cows supplemented with molasses during Winter/Fall season only. Also, year-round supplementation of molasses and range cubes will modify calf development during pregnancy, and increase calf health and growth following birth.

In June 2017 (day 0 of the study), mature Brangus cows were allocated bahiagrass pastures (84 pairs/year). Treatments consisted of control cows receiving no supplementation before calving, and then supplemented with molasses from calving (November 2017) until end of breeding season (April 2018; **MOL-W**), or cows receiving year-round supplementation of molasses (**MOL-Y**; June 2017 to May 2018) or year-round supplementation of range cubes (**CUB-Y**; June 2017 to May 2018). Total annual amount of supplement will be the same for all treatments (272 kg of supplement/cow annually; Table 5). The composition of range cubes varies throughout the year, but the main ingredient in all formulations is wheat middlings (>60% of total dry matter). Supplements are being offered twice weekly (Mondays and Thursdays) and were formulated to provide similar amounts of energy and protein. Trace mineral/vitamin supplementation is being provided during the entire year in a loose meal form for control cows, or mixed into the molasses or cubes for cows assigned to year-round supplementation (50 g/day of mineral/vitamin mix).

Table 5. Supplement daily dry matter intake of cows offered molasses during Fall/Winter only (MOL-W) or year-round supplementation of molasses (MOL-Y) or range cubes (CUB-Y).

Treatments	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
	<i>kg of supplement dry matter/cow daily</i>											
Year-round molasses (MOL-Y)	0.23	0.23	0.68	0.68	0.68	1.14	1.36	1.36	1.14	1.14	0.23	0.23
Year-round cubes (CUB-Y)	0.23	0.23	0.68	0.68	0.68	1.14	1.36	1.36	1.14	1.14	0.23	0.23
Fall/Winter Molasses (MOL-W)	0	0	0	0	0	1.82	1.82	1.82	1.82	1.82	0	0

Partial results: Molasses or range cubes supplementation was not sufficient to improve the body condition score change of cows from day 0 to 74 (June to August) compared to cows receiving no supplementation (Table 6). However, range cubes supplementation increased plasma concentrations of IGF-1 and glucose on day 56 compared to cows receiving no supplementation. Insulin-like growth factor 1 (IGF-1) and glucose are crucial for proper calf development during gestation, suggesting that although cow growth performance was not impacted, the energy status of cows was altered and that calf growth during gestation might have been impacted.

Table 6. Growth performance of cows assigned to receive no supplementation until calving, and then molasses supplementation during Fall/Winter only (November 2017 to April 2018; MOL-W), and cows offered year-round supplementation of molasses (MOL-Y) or year-round supplementation of range cubes (CUB-Y).

Item	Treatments			SEM	P-value	
	MOL-W	MOL-Y	CUB-Y		Treatment	Treatment x day
Cow body condition score (BCS) ¹						
day 56 (July 31, 2017)	5.02 ^a	5.05 ^a	5.16 ^a	0.076	<0.0001	<0.0001
day 74 (August 18, 2017)	4.92 ^a	4.85 ^a	4.96 ^a	0.076		
day 122 (October 5, 2017)	5.55 ^a	5.92 ^b	6.00 ^b	0.076		
day 161 (November 13, 2017)	5.18 ^a	6.06 ^b	5.92 ^b	0.085		
Cow BCS change						
day 0 to 56	0.55	0.56	0.66	0.079	0.61	
day 56 to 74	-0.12	-0.20	-0.18	0.069	0.66	
day 74 to 122	0.64 ^a	1.07 ^b	1.02 ^b	0.094	0.08	
day 122 to 161	-0.37 ^a	0.10 ^b	-0.08 ^b	0.107	0.01	
Cow Body Weight ¹ , kg						
day 56	427 ^a	424 ^a	440 ^b	5.4	0.32	<0.0001
day 74	403 ^a	407 ^{ab}	415 ^b	5.4		
day 122	541 ^a	545 ^{ab}	554 ^b	5.4		
day 161	541 ^a	560 ^b	534 ^a	5.4		
Plasma measurements						
Glucose on day 56, mmol/L	3.80 ^a	4.79 ^b	4.71 ^b	0.307	0.06	
IGF-1 on day 56, ng/mL	40.6 ^a	48.2 ^b	38.7 ^a	2.46	0.10	

^{a-b} Within a row, means without a common superscript differ ($P \leq 0.05$).

¹ Covariate-adjusted to cow body weight and BCS obtained on d 0 ($P \leq 0.05$).

The reason for comparing isocaloric and isonitrogenous supplements is to evaluate the impact of supplement carbohydrate composition on physiological parameters and performance of cows and their offspring. Sucrose is the main carbohydrate of molasses (Pate, 1983), whereas soluble fiber (including pectin) is the primary carbohydrate in most plant-based supplements (NRC, 2016). According to Royes et al. (2001), cattle supplemented with a high-fiber supplement have greater total ruminal VFA concentrations compared with cattle supplemented with molasses. In addition, several studies reported that feeding diets with high molasses or sucrose inclusion rates resulted in increased butyric acid concentration in the rumen of cattle (Hatch and Beeson, 1972), whereas diets rich in pectin produced a greater proportion of acetic acid (Schaibly and Wing, 1974). Butyric and acetic acid are both oxidized for energy production (Bergman, 1990), but butyrate may have a detrimental effect on VFA metabolism by restraining hepatic propionate utilization and partially inhibiting glucose synthesis, availability, and absorption (Aiello et al., 1989). Although plasma glucose concentrations did not differ on day 56 between cows supplemented with molasses or cubes, additional cow blood samples are being collected to evaluate multiple

indicators of energy metabolism of cows during the study, which will assist on improving future decisions regarding the nutritional management of these cows.

The amount of molasses and cubes supplement was increased to 0.68 kg of dry matter per cow daily from day 74 to 161 (August to October), which significantly impacted cow growth performance. Molasses and range cubes supplementation increased cow body condition score on days 122 and 161 compared to cows receiving no supplementation (Table 6). Most cows started calving around day 161. Hence, a relatively small amount of supplementation during late gestation (0.68 kg/day of molasses or range cubes dry matter) improved the nutritional status of cows during late gestation and led to better body condition score at the time of calving. It is expected that such improvement in body condition score of cows at the time of calving will increase the reproductive performance of cows during the next breeding season and calf development during late gestation, which might increase calf performance after birth. It is important to realize that cows receiving no supplementation lost body condition score during the last half period of late gestation (day 122 to 161), whereas cows supplemented with molasses or cubes were able to maintain or slightly increase the body condition score during this period. As described previously, a short-term period of energy restriction (last 40 days of gestation) did not impact the subsequent reproductive performance of cows, but had detrimental impacts on the response to vaccination and antibody production after weaning of Angus calves (Moriel et al., 2016). After calving, we will evaluate the immunity and growth performance of all calves. Steers will be sent to a feedlot for finishing and carcass data collection, and heifers will be developed until the end of their first breeding season.

STUDY #2: Evaluating Cost-effective Supplementation Programs for Cows During Late-gestation

Figure 5 represents the daily energy requirement of a mature Brangus cows calving in November and weaning its calf in July. Within a production cycle, nutrient demand of cows achieves the lowest levels during the first 30 days after calf weaning, then exponentially grows during late-gestation. This occurs because approximately 2 thirds of calf fetus growth occur during the last 90 days of gestation. However, late-gestation also corresponds with the beginning of Fall/Winter seasons when forage nutritional value and availability are low. Several cow-calf operations do not provide any kind of supplementation before calving, and inadequate dietary energy/protein during late pregnancy lowers reproduction even if the amount of energy and protein consumed after calving are sufficient (Perry et al., 1991).

Recent studies have shown that the supplementation of energy and protein during the entire-late gestation can improve postnatal offspring growth and health. It is important to highlight that most of the studies mentioned above were conducted with *Bos taurus* cows grazing cool-season forages, and not with cows having *bos indicus* genetic influence and consuming low-quality, warm-season forages that represent the majority of pastures in FL. It is unknown if cows and calves will experience similar results under our environment conditions. Hence, study #2 will: (1) evaluate if supplementation of Brangus cows during the entire late-gestation (August to November) will increase the reproductive success of cows, calf development during gestation and performance after birth to levels higher than the cost of this supplementation strategy, and

(2) investigate if concentrating supplementation during the period of lowest nutrient demand (first 6 weeks after weaning) will be more cost-effective than supplementing during the entire late-gestation.

Our group believes that cows supplemented during late-gestation, regardless of length of supplementation, will have greater profitability than non-supplemented cows due to improvements on cow reproduction and calf performance. Second, we believe that supplementing **2 kg day for 6 weeks after weaning (day 0 to 45, relative to weaning; 84 kg per cow)** will reduce labor costs by half, have the greatest improvement on cow weight gain and reproduction success, but not cause fetal-programming effects (due to the shorter supplementation period), whereas the supplementation of **1 kg/day for 12 weeks after weaning (day 0 to 84, relative to weaning; 84 kg per cow)** will have greater labor costs, have a lower improvement on reproduction, but enhance calf development during gestation and performance after birth.

Partial results: Cows receiving 1 kg/day of DDG from day 0 to 84 had greater body condition score on days 45 and 84 compared to cows receiving no supplementation (Table 7). This improved nutritional status during late gestation is expected to increase subsequent reproductive performance of cows and postnatal calf performance. In agreement with our hypothesis, cows supplemented with 2 kg/day of DDG from day 0 to 45 had the greatest body condition score on days 45 and 84. These results indicate that by concentrating the total amount of supplementation during the period of lowest nutritional requirements of cows (first 45 days after weaning), producers will harvest a greater improvement on cow body condition score and reduce labor by half compared to providing smaller amounts of supplement for a longer period.

Similar to study #1, cows receiving no supplementation (NOSUP cows) lost body condition score during the last half period of late gestation (day 45 to 84), whereas cows supplemented with 1 kg/day of DDG from day 0 to 84 increased their body condition score, which might have different outcomes to postnatal calf immunity as reported by Moriel et al. (2016). Surprisingly, cows that received 2 kg/day of DDG supplementation from day 0 to 45, but no supplementation from day 45 to 84, also increased their body condition score from day 45 to 84. Reasons for this response are not known yet. By the time this article was prepared, reproductive performance of cows and calf postnatal performance were not available. Similar to study 1, we will evaluate the immunity and growth performance of all calves after birth. Steers will be sent to a feedlot for finishing and carcass data collection, and heifers will be developed until the end of their first breeding season.

Table 7. Growth performance of cows that received no supplementation (NOSUP), and cows that were supplemented with 2 kg/day of DDG for 6 weeks after weaning (day 0 to 45; SUP6) or with 1 kg/day of DDG for 12 weeks after weaning (day 0 to 84; SUP12).

Item	Treatment			SEM	P-value	
	NOSUP	SUP6	SUP12		Treatment x day	Treatment
Cow Body Weight, kg						
day 45 (October 5, 2017)	553 ^a	582 ^b	576 ^b	5.4	0.007	0.0005
day 84 (November 13, 2017)	554 ^a	584 ^b	573 ^b	5.5		
Cow body condition score (BCS)						
day 45	5.61 ^a	6.15 ^c	5.81 ^b	0.087	<0.0001	<0.0001
day 84	5.29 ^a	6.36 ^c	6.16 ^b	0.090		
Cow BCS change						
day 0 to 45	0.53 ^a	1.04 ^c	0.74 ^b	0.096		0.0001
day 45 to 84	-0.31 ^a	0.22 ^b	0.37 ^b	0.129		0.0001

^{a-b} Within a row, means without a common superscript differ ($P \leq 0.05$).

¹Covariate-adjusted to cow body weight and BCS obtained on d 0 ($P \leq 0.05$).

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Can Differing Pre- and Post-Weaning Phase Nutrition Change Intake, Growth and Hasten Puberty in Holstein Heifer Calves?

Une alimentation différente avant et après le sevrage peut-elle modifier la consommation et la croissance et hâter la puberté chez les génisses Holstein?

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Abstract

The objective was to determine the effects pre- and post-weaning diets with differing energy levels have on the energy intake, growth, and insulin-like growth factor 1 (IGF-1) concentrations in Holstein heifer calves. Animals were randomly assigned to either a low (n = 14) or high (n = 14) pre-weaning diet (5 L/d or 10 L/d of pasteurized whole milk, wk 1 to 8.5), and to either a low (n = 14) or high (n = 14) ad-libitum post-weaning dry TMR diet [30% straw and 70% concentrate or 15% straw and 85% concentrate, from wk 9 to 25 of age], in a 2x2 factorial experiment. Free choice concentrate was available to all calves in the pre-weaning phase, and feed intake was recorded daily from birth to 26 wk of age. Once weekly, all calves were weighed and blood sampled. Metabolizable energy (ME) intake was increased in calves offered the pre-weaning high-plane diet from wk 1 (high= 3.38Mcal/d, low = 2.54Mcal/d, p<0.001) to 8 (high = 5.73Mcal/d, low = 4.90Mcal/d, p = 0.02). Regardless of pre-weaning treatment, offering a high plane of nutrition post-weaning increased ME intake from wk 11 (high = 9.52Mcal/d, low = 8.53Mcal/d, p = 0.003) to 25 (high = 22.05Mcal/d, low = 17.38Mcal/d, p < 0.001). Average daily gain (ADG) was calculated on a monthly basis, and rate of gain differed between treatments only in month 1 (high= 0.80 kg ADG, low = 0.49 ADG, p = 0.009). Serum IGF-1 concentrations, measured every other week, were greater in high-plane calves in wk 3, 5, and 7 during the pre-weaning phase. In the post-weaning phase, the same trend continued, as IGF-1 tended to be greater in high-plane calves in wk 15, 17, 19, and 21. Early life elevated planes of nutrition offer possible advantages as energy intake and IGF-1 concentrations were increased, although differences in growth were not detected.

Résumé

L'objectif était de déterminer les effets de rations pré-sevrage et post-sevrage de différentes teneurs énergétiques sur la consommation d'énergie, la croissance et l'apparition de la puberté chez des génisses Holstein. Les animaux ont été assignés aléatoirement à une ration de pré-sevrage pauvre (n = 18) ou riche (n = 18) (5 L/j ou 10 L/j de lait entier pasteurisé, semaines 1 à 8,5) et à une ration RTM sèche de post-sevrage servie ad libitum pauvre (n = 18) ou riche (n =

18) (30 % paille et 70 % concentré ou 15 % paille et 85 % concentré, semaines 9 à 25), dans le cadre d'un plan d'expérience factoriel 2x2. Un concentré a été offert en libre choix à tous les veaux durant la phase de pré-sevrage, et la consommation alimentaire a été enregistrée quotidiennement de la naissance jusqu'à l'âge de six mois. Les paramètres corporels ont été mesurés chaque semaine. Aucune différence quant à la hauteur aux hanches moyenne et à la hauteur au garrot moyenne ainsi qu'aux périmètres thoracique et abdominal n'a été observée entre les groupes de traitement durant l'étude. Des semaines 2 à 9, les veaux qui ont reçu la ration riche en énergie avant le sevrage pesaient significativement plus que ceux qui ont reçu la ration pauvre en énergie, mais cette différence est disparue après le sevrage. Un régime alimentaire intensif après le sevrage a augmenté la consommation d'énergie métabolisable comparativement au régime non intensif pour les semaines 11 (riche = 9,52 Mcal/j, pauvre = 8,53 Mcal/j, $p = 0,003$) à 25 (riche = 22,05 Mcal/j, pauvre = 17,38 Mcal/j, $p < 0,001$). À l'âge de 6 mois, un sous-groupe de génisses provenant des traitements de post-sevrage riche ($n = 11$) et pauvre ($n = 12$) ont fait l'objet d'un suivi pour confirmer la puberté par échographie transrectale. Le nombre moyen de jours jusqu'à la première ovulation a été significativement moindre chez les génisses recevant la ration de post-sevrage riche en énergie, comparativement aux veaux qui ont consommé la ration pauvre en énergie après le sevrage (201,73, écart-type = 7,76 vs 240,75, écart-type = 7,36, $p = 0,04$). L'alimentation intensive en début de vie offre de possibles avantages pour les producteurs et les animaux, considérant que, dans cette étude, la consommation d'énergie a augmenté, que l'âge à la puberté a diminué et que la croissance n'a pas été compromise.

Introduction

Developing heifer calves from birth is an important task that can significantly impact the future efficiency, production, and profitability of a farm. Successful heifer development is heavily influenced by early life nutrition. Many recent studies examining the impacts of early life nutrition have focussed on three time periods of developmental significance; the pre- weaning, post- weaning, and post- pubertal phases. Of these, the pre- weaning period is most commonly studied, as large increases in lifetime milk yield and longevity have been associated with increased average daily gains (ADG) during this time (Bach, 2011; Soberon et al., 2012; Soberon and Van Amburgh, 2013). Increases in pre- weaning ADG have mainly been achieved through providing increased volumes of milk, while increases in ADG during the less- studied post- weaning phase have generally been the result of increased energy provision from feed.

The results of increasing early-life ADG are not always conclusive, as similar research has indicated greater ADG may reduce heifer size (Van Amburgh et al., 1998), and have no effect on future milk production (Terré et al., 2009). In addition, very few studies have examined the interactions of pre- and post- weaning planes of nutrition, or lack the animal numbers necessary to do so. Therefore, our objective was to determine the effects of pre- and post- weaning diets, with differing energy levels, on the energy intake, growth, and hormone profile of Holstein heifer calves.

Methodology

Calves were randomly assigned to 1 of 4 treatment groups at birth (Figure 1). Pre- weaning dietary treatments were 5L whole milk / calf / day (L) vs 10L whole milk / calf / day (H), but all calves had *ad libitum* access to texturized starter (22.4% crude protein, 7.6% crude fibre, 2.9% crude fat) from wk 1 to 9. Following a 10-day step down weaning process concluding at day 65, *ad libitum* access to starter continued for 5 days, at which point all calves were abruptly transitioned to a dry TMR made of 85% starter and 15% chopped straw (H, 25.0% crude protein, 2.94 Mcal/kg). All calves received the H TMR for 2 weeks, and were moved to an outdoor facility, before calves assigned to the low post- weaning treatment were abruptly transitioned to a 70% starter 30% chopped straw ration (L, 21.1% crude protein, 2.49Mcal/kg) on which they remained for the rest of the trial. Free choice water was available throughout.

Pre- Post-	85% concentrate dry TMR	70% concentrate dry TMR
10L milk/day	HH	HL
5L milk/day	LH	LL

Figure 1. Dietary treatments in the pre- and post- weaning phases.

Feed intake was measured on a daily basis for the duration of the study. Once weekly, approximately 2.5 hours after the morning feeding, 2 x 10 mL blood samples were collected via jugular venipuncture for harvesting plasma and serum. Immediately following blood collection, weight and body measures were recorded. Serum IGF-1 concentrations from alternate weeks were determined using an established solid-phase, enzyme-labeled chemiluminescent immunometric assay (Immulite 1000 IGF-1, Siemens Healthcare Ltd., Oakville, ON).

Results and Discussion

As expected, ME intake was increased in H calves through most of the pre- weaning period. During this phase, the majority of ME is supplied through milk. As energy requirements increase with age, and the energy supplied through the milk remained constant, L calves were able to compensate for their energy deficit by increasing starter intake. Jasper and Weary (2002) also noted large increases in starter consumption for calves receiving a low plane of nutrition in the pre- weaning period, and limiting milk intake has traditionally been used on farm to stimulate starter intake. During weeks 9 and 10, immediately following weaning, no significant differences in energy intake were found between treatment groups. However, in weeks 11 through 25, calves receiving the H post-weaning diet had significantly greater ME intake than L post- weaning calves.

Monthly ADGs differed between treatments only in month 1 ($p = 0.095$, H = 0.80 kg/d, L = 0.49 kg/d). In the remaining 5 months no differences between treatments or interactions between pre- and post- weaning diet were detected. Mean average daily gains for the post-weaning period were also similar between groups (HH = 1.28kg/day, HL = 1.18kg/day, LH = 1.30kg/day, LL = 1.24 kg/ day). Overall, ADGs in the post-weaning phase were high, with peak gains of 1.56kg/day and 1.44kg/day being reached at month 5 by H and L post- weaning groups respectively. These large ADGs are thought to be a function of the relatively high energy diets provided to the heifers, as commonly practiced feeding regimes are planned for ADGs of approximately 0.9 kg/day post-weaning in order for heifers to be large enough to calve at 22- 24 months.

Serum IGF-1 levels were greater ($p < 0.05$) in weeks 3, 5, and 7 for H pre- weaning calves (131ng/mL, 106ng/mL, 120ng/mL) than L pre-weaning calves (77 ng/mL, 70 ng/mL, 95ng/mL respectively). These results were in agreement with previous studies, where increased IGF-1 levels were correlated with increased feeding rates, protein intake, and growth (Bartlett, 2001; Brown et al., 2005). In the post- weaning phase, no effect of pre- weaning diet was detected, but H calves again had increased IGF-1 concentrations at weeks 17, 21, and 26 (201ng/mL, 214ng/mL, 239ng/mL) compared to L calves (159ng/mL, 175ng/mL, 194ng/mL respectively). Insulin-like growth factor 1 plays a significant role in many aspects of development, and has also been discussed as a signalling hormone for puberty (Maciel et al., 2004), making it especially relevant to heifer rearing.

Conclusions

In summary, offering increased planes of nutrition in the pre- and post- weaning phase increased ME intake, and elevated IGF-1 serum concentrations. Elevated planes led to increased growth in the first month of life, but average daily gains did not differ in the remainder of the trial. Although no differences in growth were found in the post- weaning phase, future research is needed to monitor potential differences in development that may occur as a result of elevated energy intake or IGF-1 level.

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Oxidative Damage of Biological Molecules on Animal Metabolism and Physiology

Dommages oxydatifs des molécules biologiques sur le métabolisme et la physiologie des animaux

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Abstract

In commercial animal/poultry production a range of various stresses are responsible for economical losses due to deterioration of health status, disturbances of microbiota and gut integrity, decreased productive and reproductive performance of animals. There are several important periods of animal development (e.g. gestation, birth, weaning and transition period for mammals, late embryonic development and early posthatch time for birds, etc.) which are characterised by increased susceptibility to detrimental consequences of commercially-relevant stresses. It has been proven that at the molecular level nutritional, technological, environmental and internal/biological stresses are associated with overproduction of free radicals, disturbances of redox balance and oxidative stress. However, reactive oxygen species (ROS), depending on their concentration and site of production, could be detrimental or beneficial. Therefore, understanding molecular mechanisms of signalling functions of ROS and regulatory roles of various transcription factors, including Nrf2 and NF- κ B, are among major priorities in nutritional and veterinary sciences. Recently it has been suggested that adaptation to stress at the molecular level is mediated by a range of genes called vitagenes. The vitagene concept of adaptation to stress due to production of protective molecules, including heat shock proteins (HSP), antioxidant enzymes such as superoxide dismutase (SOD) and thioredoxin reductase (TR) as well as sirtuins was successfully transferred from medical sciences to avian/animal sciences and practical applications of the concept await further development. Therefore, there is a need to find optimal ways of vitagene regulation to improve animal adaptive ability to stress. This includes traditional antioxidant supplements (e.g. vitamin E and selenium) as well as new entrants into antioxidant family, including carnitine, betaine and taurine.

Résumé

Dans le secteur des productions animales commerciales, divers facteurs de stress sont responsables de pertes économiques liées à une baisse des performances de production et de reproduction. On a prouvé qu'à l'échelle moléculaire, des stress nutritionnels, technologiques, environnementaux et internes sont associés à la surproduction de radicaux libres, à la perturbation de l'équilibre redox et au stress oxydatif. Il est reconnu que le stress oxydatif entraîne un certain nombre de conséquences dommageables pour le système immunitaire, pour la santé gastro-intestinale, notamment pour l'équilibre du microbiote, ainsi que pour d'importants paramètres de croissance et de développement chez les animaux. Des systèmes antioxydants sont apparus au cours de l'évolution pour éliminer les radicaux libres et produits toxiques du métabolisme. Divers antioxydants sont synthétisés dans le corps, dont des enzymes antioxydants, le glutathion, la thiorédoxine, la coenzyme Q, etc. Cependant, en conditions de stress, les systèmes antioxydants internes ont besoin d'aide de l'extérieur, laquelle est fournie par les aliments et l'eau sous la forme d'additifs alimentaires antioxydants. La vitamine E, les caroténoïdes et le sélénium (un précurseur des sélénoprotéines) comptent parmi les principaux antioxydants issus des aliments. Les rôles antioxydants de la carnitine, de la bétaine et de la taurine ont récemment été démontrés. En effet, ces nutriments sont synthétisés dans le corps des animaux, mais on a montré qu'un apport alimentaire joue un rôle important. Mentionnons que les ingrédients alimentaires d'origine végétale sont de piètres sources de carnitine et de taurine. Compte tenu de la diminution substantielle de la quantité d'ingrédients d'origine animale utilisés dans les rations des volailles et du bétail, la carnitine et la taurine deviennent des nutriments essentiels conditionnels et un apport alimentaire, surtout en conditions de stress, pourrait aider à compenser la surproduction de radicaux libres. Comprendre les fonctions de signalisation des formes réactives de l'oxygène (FRO) et les mécanismes moléculaires des stress oxydatifs compte parmi les principales priorités en science de la nutrition et en science vétérinaire. On a récemment avancé qu'un ensemble de gènes, appelés vitagènes, interviennent dans les mécanismes d'adaptation au stress à l'échelle moléculaire. Le concept de l'adaptation au stress par vitagènes a été transféré avec succès des sciences médicales aux sciences animales, et on s'attend à ce que d'autres applications pratiques du concept soient développées. Une brève description des rôles de la vitamine E, des caroténoïdes et du Se dans la régulation des mécanismes de protection antioxydante sera présentée dans l'exposé. De plus, la modulation nutritionnelle des vitagènes et les mécanismes moléculaires d'adaptation aux stress seront caractérisés. Une attention spéciale sera accordée à l'équilibre redox dans le tube digestif et à son influence probable sur le microbiote gastro-intestinal. Les rôles possibles des vitagènes dans l'immunomodulation des animaux d'élevage seront considérés.

Introduction

In commercial animal production a range of various stresses are responsible for economical loses due to decreased productive and reproductive performance of farm animals. It has been proven that at the molecular level nutritional, technological, environmental and internal stresses are associated with overproduction of free radicals, disturbances of redox balance and oxidative stress (Surai and Fisinin, 2016a; b). It has been established that oxidative stress has a number of

detrimental consequences affecting immune system, gut health, including microbiota disbalance, as well as major parameters of animal growth and development.

Free radicals and reactive oxygen and nitrogen species

Free radicals are atoms or molecules containing one or more unpaired electrons. Free radicals are highly unstable and reactive. They are capable of damaging biologically relevant molecules such as DNA, proteins, lipids or carbohydrates. It is believed that the animal body is under constant attack from free radicals, produced as a natural consequence of the body's normal metabolic activity and as part of the immune system's strategy for destroying invading microorganisms. Collective terms ROS and reactive nitrogen species (RNS) have been introduced (Halliwell and Gutteridge, 1999) and they include not only the oxygen or nitrogen radicals, but also some non-radical reactive derivatives of oxygen and nitrogen. The most important effect of free radicals on the cellular metabolism is due to their participation in lipid peroxidation reactions (Surai, 2006). Therefore, lipid peroxidation is a chain reaction and could cause substantial damage to cells. It is generally accepted that polyunsaturated fatty acids (PUFA), responsible for maintenance of physiologically important membrane properties including fluidity and permeability, are easily oxidised. In fact, their susceptibility to peroxidation is proportional to number of double bounds in the molecules. Furthermore, PUFA are known to be involved in numerous biological processes and play crucial roles in various cellular functions, including cellular barriers, signaling, energy storage, growth and survival. Therefore, lipid peroxidation is closely associated with various disease states, decreased productive and reproductive performance in farm animals and poultry (Surai, 2002, 2006). Lipid peroxidation produces a wide variety of oxidation products. The main primary products of lipid peroxidation are lipid hydroperoxides (LOOH). Among them, malondialdehyde (MDA) appears to be the most mutagenic product of lipid peroxidation, whereas 4-hydroxyalkenal (4-HNE) is the most toxic one (Ayala et al., 2014). Indeed, 4-HNE is highly reactive toward nucleophilic thiol and amino groups and could form covalent adducts with various cellular (macro)molecules, including lipids, proteins, and nucleic acids. This leads to various detrimental consequences of cellular structure and metabolism, including inhibition of protein and DNA synthesis, dysregulation of enzyme activities, alteration in mitochondrial coupling, etc. (Hu et al., 2017). Therefore, major systems of the animal body, including cardiovascular system, reproductive system, immune system, liver and kidney are affected due to lipid peroxidation. Lipid peroxides are shown to exert their toxic effects through two important mechanisms. Firstly, lipid peroxidation is associated with alterations in the assembly, composition, structure, and dynamics of lipid membranes leading to detrimental consequences in cell functions. Secondly, lipid peroxides could promote further generation of new ROS with formation of new reactive compounds capable of damaging/crosslinking DNA and proteins (Gaschler and Stockwell, 2017). Lipid peroxidation also plays a role in regulated cell death. For example, the lipid degradation product 4-HNE has been shown to induce apoptosis in specific contexts and lipid peroxidation is the primary driver of ferroptosis, a type of regulated necrotic cell death (Gaschler and Stockwell, 2017). Furthermore, 4-HNE can be found at low concentrations in human tissues and plasma and participates in the control of biological processes, such as signal transduction, cell proliferation, and differentiation (Pizzimenti et al., 2013). From an analytical point of view, quantitative determination of MDA in plasma, urine and other biological samples is easier than that of HNE and MDA and HNE are shown to correlate

closely with each other. However, reliable measurement of MDA in biological samples is quite challenging and requires special precautions at the pre-analysis stage (Tsikas et al., 2017).

Proteins and DNA are also important targets for ROS. The complex structure of proteins and a variety of oxidizable functional groups of the amino acids make them susceptible to oxidative damage. Indeed, proteins exposure to ROS causes modification of amino acid side chains and alteration of the protein structure leading to functional changes disturbing cellular metabolism associated with several pathological states (Ahmad et al., 2017). In fact, increased side-chain hydrophilicity, side-chain and backbone fragmentation, aggregation via covalent crosslinking or hydrophobic interactions, protein unfolding and altered conformation, altered interactions with biological partners and modified turnover are observed due to protein oxidation (Davis, 2016). The accumulation of oxidized proteins has been implicated in a range of age-related pathologies. Furthermore, a range of oxidized proteins and amino acids has been characterised in biological systems (Kehrer, 2000). In general, the accumulation of oxidized proteins depends on the balance between antioxidants, prooxidants and removal/repair mechanisms and leads to the formation of reversible disulfide bridges. More severe protein oxidation causes a formation of chemically modified derivatives e.g. Schiff's base (Tirosh and Reznick, 2000). Interestingly, protein peroxides can oxidize both proteins and other targets (Davis, 2016). Nitric oxide, hydroxyl radical, alkoxyl and peroxy radicals as well as carbon-centered radicals, hydrogen peroxide, aldehydes or other products of lipid peroxidation can attack protein molecules. Usually oxidative modification of proteins occurs by two different mechanisms: a site-specific formation of ROS via redox-active transition metals and non-metal-dependent ROS-induced oxidation of amino acids (Tirosh and Reznick, 2000). The modification of a protein occurs by either a direct oxidation of a specific amino acid in the protein molecule or cleavage of the protein backbone. In both cases biological activity of the modified proteins would be compromised. The degree of protein damage depends on many different factors (Grune et al., 1997):

- the nature and relative location of the oxidant or free radical source;
- nature and structure of protein;
- the proximity of ROS to protein target;
- the nature and concentrations of available antioxidants.

It seems likely that direct oxidation of cysteine and methionine residues in proteins are major reactions due to oxidative stress and this results in altered protein activity and function (Davis, 2016).

It has been shown that the DNA in each cell of a rat is hit by about 100,000 free radicals a day and each cell sustains as many as 10,000 potentially mutagenic (if not repaired) lesions per day arising from endogenous sources of DNA damage (Ames and Gold, 1997; Helbock et al., 1998; Ames, 2003; Diplock, 1994). Therefore, some oxidative lesions escape repair and the steady state level of oxidative lesions increased with age, and an old rat has accumulated about 66,000 oxidative DNA lesions per cell (Ames, 2003). Oxidation, methylation, deamination and depurination are four endogenous processes leading to significant DNA damage with oxidation to be most significant one and approximately 20 types of oxidatively altered DNA molecules have been identified. The chemistry of attack by ROS on DNA is very complex and lesions in

chromatin include damage to bases, sugar lesions, single strand-breaks, basic lesions and DNA-nucleoprotein cross-links (Diplock, 1994). DNA damage is thought to contribute to carcinogenesis, aging, and neurodegenerative diseases through mutations, genome instability, and perturbed signaling. Furthermore, lipid peroxidation-derived aldehydes and their exocyclic DNA adducts could be implicated in mutations (Kawai and Nuka, 2018). Possible roles of DNA oxidation/damage in health maintenance, productive and reproductive abilities of farm animals await further investigation.

In general, oxidative stress in farm animals and poultry and damages to biological molecules are associated with compromised health status including immunosuppression, decreased productive and reproductive performance (Surai, 2002; 2006; Surai and Fisinin, 2016a; 2016b). It is important to mention that depending on type and concentration ROS can play positive or negative roles in cellular physiology and ROS are thought to be essential consequences and drivers of evolution and survival over Earth history (Taverne et al., 2018). Furthermore, ROS generation is an important evolutionarily conserved process playing essential role in the cell signaling mechanism as well as the cellular defense mechanism against microbial invasion (He et al., 2017).

Stresses in poultry production

From a physiological point of view, stress is related to a deviation from optimal internal and external conditions. Oxidative stress-related nutritional metabolic diseases (e.g. encephalomalacia, exudative diathesis, muscular dystrophy, etc.) in modern commercial poultry production practically disappeared (Surai, 2002; 2006). However, oxidative stress-related various disorders of the biological antioxidant defence system still causing substantial problems. This is related to imbalanced diets or disturbances in their assimilation or metabolism due to various environmental factors (Mezes et al., 1997). It seems likely that a long selection for improvement of chicken growth made them particularly susceptible to oxidative stress (Soleimani et al., 2011). Four major types of stress in poultry industry are shown can be considered, including technological, environmental, nutritional and internal stresses (Surai and Fisinin, 2016a; b).

From one hand, environmental stresses started from the moment when egg is laid, since temperature variation could cause embryo to start developing (high environmental temperature) or die (low temperature or fast temperature change; Surai and Fisinin, 2016a). Furthermore, temperature and other conditions of egg storage (e.g. humidity) between egg laying and its placement into the hatchery could negatively affect embryonic development. It was shown that hatchability of fertile eggs declines depending on length of storage with a significant increase in percentages of early and late embryonic mortality with increasing length of storage period (Fasenko, 2007) and this, most likely, could affect chickens in later life. On the other hand, chicken hatch is considered to be associated with oxidative stress. Indeed, high temperature and humidity as well as high proportion of PUFA in the tissue lipids predispose chicken embryo to oxidative stress at time of hatching (Surai et al., 1996). Chick viability is an important factor determining poultry production profitability and there is a range of factors such as egg quality, incubation conditions and post-hatch environment affecting chick quality (Decuypere et al., 2001). It is proven, that time between chick hatch and placement, which could comprise up to 36

h, is major technological stress. Therefore, there is an inverse relationship between duration of post-hatching holding time and subsequent chick viability and performance (Fisinin and Surai, 2012a; 2012b). Our data clearly indicate that extended time in the hatcher (36h) caused a significant decrease in antioxidant defences system as indicated by decreased vitamin E and coenzyme Q concentrations in chicken tissues (Karadas et al., 2011). Taking into account the relatively high temperature and humidity in the hatcher, it is obvious that the chick may be under chronic oxidative stress during this holding time (Fisinin and Surai, 2012; 2012a). Therefore, antioxidant protection at hatching time is considered to be main determinant of chick viability in early postnatal development (Surai, 2002; Surai et al., 1998; 1999; 1999a; Surai et al., 2016). It seems likely that during chick embryo development an antioxidant/prooxidant (redox) balance in the tissues supports normal embryonic development and post-hatch chick viability (Surai et al., 1996; Surai and Fisinin, 2015). It has been shown that an accumulation of the natural antioxidants (vitamins A, E and carotenoids) as well as an increase in glutathione peroxidase (GSH-Px) activity in the embryonic liver may have an adaptive mechanism responsible for protection of unsaturated lipids against peroxidation during the stress imposed by hatching (Surai, 2002; Surai et al., 2016).

Early postnatal nutrition is a critical factor for the developmental maturation of many systems and optimal physiological functions. In fact, there is a growing body of evidence indicating that nutritional exposures during these critical and sensitive periods of life can cause permanent changes in many physiological processes, which are known as “programming” (Amarasekera et al., 2013). For example, our data indicate that low quality neonatal nutrition during short period of time resulted in long-term impairment in the capacity to assimilate dietary antioxidants in birds (Blount et al., 2003). It could well be that early programming associated with epigenetic mechanisms plays a significant role in chicken growth and development. Furthermore, the programming effects of conditions during early development could be transmitted to the offspring (Champagne and Rissman, 2011). It seems likely that transgenerational effects of stress are mediated via modulation of the hypothalamic–pituitary–adrenal axis as well as epigenetic mechanisms leading to heritable changes in gene expression and early experiences may shape phenotypes of chickens in a long-term way (Goerlich et al., 2012).

Oxidative stress and mammalian reproduction

A growing body of evidence indicate that the antioxidant-prooxidant (redox) balance is an important regulator of the mammalian reproductive functions, including ovarian follicular development, ovulation, fertilization, luteal steroidogenesis, endometrium receptivity and shedding, embryonic development, implantation and early placental growth and development (Al-Gubory et al., 2010). In fact, ROS have a dual role in the female reproduction: they serve as key signal molecules in physiological processes but can also be considered important elements in pathological processes (Rizzo et al., 2011). It was shown that oxidative stress is a major player in the pathophysiology of many different disorders, including complications of gestation (Burton and Jauniaux, 2011). For example, similar to chicken embryonic development, gestation is considered to be associated with oxidative stress arising from increased placental mitochondrial activity and production of ROS. Interestingly, the placenta also produces ROS affecting placental function including trophoblast proliferation and differentiation and vascular reactivity (Myatt and

Cui, 2004). Therefore, the antioxidant defence system is shown to be extremely important in animal reproduction (Surai, 2006; Surai and Fisinin, 2015).

Oxidative stress and antioxidant defences in sows

A sow reproduction stress hypothesis has been recently proposed (Wen et al., 2009). The authors suggested that the important reproductive tasks such as estrus, gestation, birth and lactation cause the reproductive stress. In particular, gestation is considered as constant oxidative stress for the dam (Wisdom et al., 1991). It seems likely that at time of gestation sows are characterised by comparatively low antioxidant defences associated with a decline in serum vitamin E from about 80 to 90 days after insemination through farrowing (Mahan et al., 2007) and decreased plasma vitamin E concentration at the end of gestation (d 110 of gestation) as compared with d 30 of gestation (Berchieri-Ronchi et al., 2011). This was associated with increased lymphocyte DNA damage in the second quarter of gestation, throughout the gestational and weaning periods. Indeed, the reproductive performance of sows was shown to be related to their oxidative stress status during gestation and lactation (Zhao et al., 2013) with a risk of compromised growth and health of foetuses as well as postpartum growth of piglets (Zhao et al., 2013). In addition, the sow ascorbic acid status is shown to be low during the period of time of extensive stress and serum Se and GSH-Px activity declined around day 60 post-coitum and decreases even more rapidly as approaching parturition (Mahan et al., 2007)

It is generally accepted that environmental stress is responsible for compromised antioxidant system of the sow. In fact, increased oxidative damage to lipid, protein, and DNA was shown to be the major contributing factors for reduced reproductive performance of sows due to heat stress (Zhao et al., 2011). Another stress caused by inclusion of fish oil (2%) into the diet lead to an oxidative stress and increases lipid peroxidation in sows (Tanghe et al., 2015). Similarly, increasing amounts of fish oil in the diet lead to decreased oxidative stability of erythrocytes and reduced both piglet survival and weaning weight with increasing quantities of fish oil supplemented (Cools et al., 2011). It seems likely that maternal nutrition may alter reproductive function of male offspring (Mack et al., 2014) and oxidative stress in gestating sows is an important risk factor of decreased sow productive and reproductive performance and could detrimentally affect progeny.

Oxidative stress and antioxidant defence in newly born piglets

It is well appreciated that during the foetal-neonatal transition and at birth important circulatory and respiratory changes associated with an increase in the O₂ partial pressure in tissues occur causing a physiological oxidative stress (Sastre et al., 1994). Therefore, newborn mammals, similar to newly hatched chicks, are at high risk of oxidative stress being very susceptible to free radical oxidative damage. Important predisposing factors include exposure to high oxygen concentrations, reduced antioxidant defence and presence of free iron which enhances the Fenton reaction leading to production of highly toxic hydroxyl radicals (Saugstad, 2005). Indeed, a compromised redox balance (an imbalance between antioxidant- and oxidant-generating systems) leads to oxidative damages (Buonocore et al., 2001) and newborn piglets are shown to

suffer seriously from birth oxidative stress due to the immature antioxidant system (Yin et al., 2013). Indeed, during mammalian prenatal development, the antioxidant system is found to be as immature (Fantel, 1996) with respective maturation in the postnatal life. For example, it was shown that in the liver of newborn rats GSH-Px and glutathione transferase (GST) activities represent only 20% and 15% of the values in the adult animals (Pallardo et al., 1991). Furthermore, activities of two major antioxidant enzymes namely SOD and GSH-Px in the piglet plasma and ileal Nrf2 significantly increased during the first week after birth preventing lipid and protein oxidation in piglet plasma (Yin et al., 2013).

Oxidative stress and immunity

If we imagine that immune system is an army fighting against invaders (microorganisms, viruses, etc.) than we would expect them to have something like mobile phones to receive and send signals to each other. Remarkably enough, major immune cells (macrophages, neutrophils/heterophils, T- and B-lymphocytes) have on their surface something like “mobile phones” called receptors. Those receptors are extremely sensitive to communicating molecules, but they are also sensitive to free radicals and can be easily damaged. In such a situation without proper communication all those huge armies of immune cells would become useless. They also can start fighting each other as well, eventually destroying immunocompetence and causing autoimmune reactions. If we imagine that immune cells are soldiers using chemical weapon to kill enemy, then special ammunition protecting them from their own weapon would be a crucial for effective battle. In the case of immune cells such ammunition is represented by natural antioxidants with Se-GSH-Px, thioredoxin reductase and other selenoproteins being major defences. Indeed, if not properly protected, macrophage functions could be compromised including initial overproduction of free radicals with consecutive damages to specific enzymatic systems resulting in decreasing efficiency of oxidative burst and apoptosis. Therefore, it is clear that antioxidant defence is a crucial factor of immune defence in the body. The aforementioned model was first introduced in 2002 (Surai, 2002) and now there is a great body of information confirming this idea. In fact, recently in a comprehensive review (Lugrin et al., 2014) it has been proven that oxidative stress affects all stages of the immune/inflammatory response, including the release by damaged tissues of signaling molecules acting as endogenous danger signals, their sensing by innate immune receptors from the Toll-like (TLRs) and the nucleotide-binding and oligomerization domain (NOD)-like (NLRs) families, with the following activation of signaling pathways initiating the adaptive cellular response to such signals. In fact, pathogen recognition receptors (PRRs) are responsible for the activation of signaling pathways leading to an inflammatory, antimicrobial response. A growing body of evidence demonstrates a link between the innate PRRs and the activation of the adaptive immune response responsible for building an effective defense against invasive organisms (Fritz et al., 2007). Moreover, it seems likely that PRRs are sensing not only invading pathogens, but also recognise endogenous nonmicrobial “danger” or stress signals, resulting in the activation of inflammatory signaling pathways including nuclear factor NF- κ B and mitogen-activated protein kinases (MAPKs; Chen et al., 2009). Interestingly, receptors play a major role in signalling leading to apoptosis, a major cause of immune cell number reduction in stress conditions. Indeed, apoptosis was shown to be triggered by three main signalling pathways, upstream of caspase activation, including an

extrinsic pathway involving death receptors localised at the cell surface, or intrinsic pathways involving mitochondria or the ER (Redza-Dutordoir and Averill-Bates, 2016).

Oxidative stress in ruminants

Despite substantial achievements in dairy and beef cattle industries for the last 30 years there are several problems which need to be solved. It seems likely that three weakest links there include: immunity (high somatic cell counts, mastitis, etc.), reproduction (low conception rate, retained placenta, metritis, cystic ovaries, etc.) and viability in early postnatal life (calf death loss at birth or between birth and weaning is a significant source of reduced reproductive rate, Surai, 2006). The increased incidence of health problems observed during the periparturient period can be partly attributed to suboptimal immune responses due to production and environmental stresses (Aleri et al., 2016), however, molecular mechanisms regulating those changes are not well defined. This is a transition from non-lactating pregnant status to non-pregnant lactation status, a period critically important to health, production and profitability of dairy cows. Indeed, most infectious diseases and metabolic disorders including retained placenta, metritis and mastitis take place during this time. The physical and metabolic stresses of pregnancy, calving, and lactation may contribute to this decrease in host resistance and the subsequent increase in disease incidence (Goff and Horst, 1997). Indeed, substantial evidence indicates that innate and acquired defense mechanisms are lowest from 3 wk pre-calving to 3 wk post-calving. This leads to the increased incidence of peripartum diseases including retained placenta, increased somatic cell counts and mastitis (Surai, 2006).

Retained placenta (retained foetal membranes, RFM)

Retained placenta (RP) where the fetal placenta is not expelled within 8-12 hr after calving, is a reproductive abnormality of the cow with the incidence varying from 3 to 39% of parturition (Kimura et al., 2002). Indeed, RP has remained a problem for dairy industry affecting about 3.6% cows in the United Kingdom (Esslemont and Kossaibati, 1996), 9% in USA (Kellogg et al., 2001) or up to 24.9% in Egypt (Gaafar et al., 2010). RP results in considerable economic loss due to decreased milk production, longer interval to first oestrus and breeding; increased number of services per conception; lower conception rate and higher incidence of metritis and increased culling rate (Attupuram et al., 2016). It is unclear why some cows fail to expel the placenta following calving. Both the mechanism of placental expulsion and the cause of RP remain unclear (Haeger et al., 2016). Various factors including age, species, heredity, environment, hormones and nutrition have been considered to be involved in the development of RP. It was suggested that immunosuppression at calving is related to problems of expulsion of fetal membranes. Therefore, RP is related to failure of the immune system to break down the placentome promptly after delivery of foetus (Kimura et al., 2002). It seems likely that impaired neutrophil function causes retained placenta. For example, neutrophils isolated from blood of cows with RP had significantly lower neutrophil function before calving, and this impaired function lasted for 1 to 2 wk after parturition (Kimura et al., 2002). A loss in neutrophil chemoattraction for fetal membrane tissue after parturition and decreased superoxide radical production by neutrophils were observed in those cows that would develop metritis and

characterised by RP (Cai et al., 1994). It is well accepted that neutrophils are the primary mechanisms of uterine (Bondurant, 1999) and mammary (Mallard et al., 1998) immune defence.

Increased SSC

Mammary gland health is an important issue in dairy industry and intramammary infections are responsible for decreased milk production and compromised milk quality. Milk somatic cells include several cell types, including neutrophils, macrophages, polymorphonuclear lymphocytes and smaller percentage of epithelial cells of the mammary gland. In the healthy lactating mammary gland, total somatic cell count (SCC) is often <100,000 per ml of milk (Sordillo et al., 1997) and increases in SCC levels in raw milk are associated with mastitis, an inflammatory reaction of the mammary gland most often due to bacterial infection (Murphy et al., 2016). SSC is used as an important indicator of udder health since SCs participate in protecting the mammary gland from infection as part of the natural (innate) immune system (Li et al., 2014). Strategies for lowering somatic cell count include breeding, husbandry, hygiene, milking technique and nutrition. It seems likely that optimal nutrition is of major importance to maintain general health and low SCC. Therefore, in addition to correct feeding, dietary supplementation with selenium and vitamin E is recommended to reduce the incidence of mastitis and thus somatic cell counts (Baltay and Bedo, 2000).

Mastitis

Mastitis, or inflammation of the mammary gland, represents another expensive problem for dairy farmers and is recognised as the costliest disease facing the world's dairy industry (Dingwell et al., 2003). The annual economic losses due to mastitis in India, United States, UK and worldwide have been estimated at \$1.1 billion, \$2 billion, \$371 million and \$35 billion respectively (Mushtaq et al., 2018). Factors predisposing cattle to mastitis are categorised as animal factors and environmental factors (Spain, 2005). Animal factors are related to anatomical structure and physiological functions of mammary gland and teats. In relation to the metabolic factors it was admitted that the incidence of mastitis occurred in two distinctive periods of the dry period. These are the first three weeks after dry off and last two weeks of gestation and early lactation. The common pathogens causing mastitis include *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus agalactiae* and other streptococci (Bassel and Caswell, 2018). Mastitis incidence is probably related to the decreased efficiency of immune system at these periods of ontogenesis. For example, the proportion of CD62L⁺ neutrophils decreased significantly at calving (Meglia et al., 2001). In fact, these changes at calving may result in less migration of blood neutrophils into the tissues and might contribute to the increased susceptibility to infections at this time. Therefore, in beef and dairy cattle transition periods are associated with markedly increased susceptibility to mastitis due to alterations in their neutrophil functions (Bassel and Caswell, 2018). It is interesting to note that higher producing cows are at greater risk of mastitis (Spain, 2005). Indeed, cows with higher milk yield in the previous lactation are more likely to have retained placenta, early metritis, silent heat, ovarian cysts, and other infertility than are cows with lower milk yield (Grohn and Rajala-Schultz, 2000). Furthermore, it was shown that mastitis is associated with decreased pregnancy rates, aberrations in the oestrous cycle, early embryonic

mortality or abortions, prolonged days open, higher number of services per conception and decreased conception rate in dairy cattle (Kumar et al., 2017).

Antioxidant systems in animal body

Antioxidant systems have been developed during evolution to deal with free radicals and toxic products of their metabolism. There is a range of antioxidants synthesised in the body, including antioxidant enzymes, glutathione, thioredoxin, coenzyme Q, etc. (Surai, 2002). However, in stress conditions internal antioxidant systems need external help which is provided via feed/water in the form of antioxidant feed additives. Vitamin E, carotenoids and selenium (as a precursor of selenoproteins) are among major feed-derived antioxidants (Surai, 2006). Recently carnitine and taurine have been shown to play antioxidant roles at the cellular/subcellular levels. Indeed, these nutrients are synthesised in animal body, but their dietary provision is shown to play a major role. It should be mentioned that plant feed ingredients are poor sources of carnitine and taurine. Due to a substantial reduction of usage of animal feed ingredients in poultry/animal diets carnitine and taurine become conditionally essential nutrients and their dietary supply, especially in stress conditions, could help deal with overproduction of free radicals (Surai, 2015b; 2015c). Antioxidant defence mechanisms in the body are summarised in Figure 1.

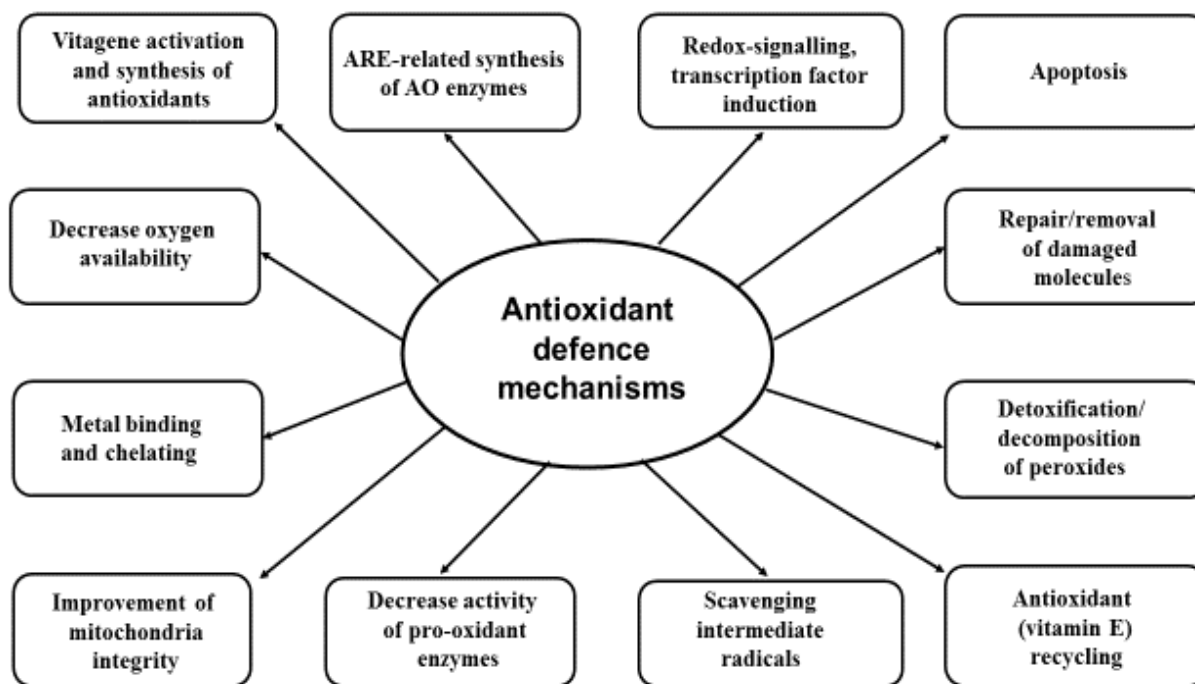


Figure 1. Antioxidant defence mechanisms (adapted from Surai et al., 2017a).

Oxidative Stress and Transcription Factors

It is important to mention that ROS are no longer viewed as just toxic by-products of mitochondrial respiration but are now appreciated for their role in regulating a great number of cellular signaling pathways. In fact, oxidation-reduction (redox) based regulation of gene

expression is a fundamental regulatory mechanism in cell biology acting at the cell-signaling level. Indeed, the field of redox signaling that is the overlap of signal transduction with redox biology is quickly developing. It is well appreciated that redox signaling is essential in maintaining physiological homeostasis and deviation from redox homeostasis results in compromised immunity/disease development (Forman, 2016), decreased productive and reproductive performance of farm animals and poultry. Therefore, in animals, redox-signaling pathways use ROS as signaling molecules to activate genes responsible for regulation of various functions including growth, differentiation, proliferation and apoptosis. Furthermore, the antioxidant defence systems are also under regulation by various transcription factors. In fact, redox balance is controlled by a battery of transcriptional factors, including Nrf2, NF- κ B, PPARs, PGC-1 α , p53, FoxO, MAPK, AP1, etc. They regulate redox status through modulating ROS-generating enzymes and antioxidant enzymes (Nrf2) and inflammation (NF- κ B) in a cooperative and interactive way being critically important for animal adaptation to various stresses (Surai, 2015c).

Vitagene concept development

Recently vitagene concept of animal adaptation to stress has been developed (Surai and Fisinin, 2016c). In fact, the term 'vitagene' was introduced by Rattan (1998) who suggested that adaptation to stress is mediated by a complex network of several genes, which may be called 'vitagenes'. Later, the vitagene concept has been further developed and adapted to human health by Calabrese and colleagues (Calabrese et al., 2007) suggesting that the vitagene family includes:

- Heat shock proteins (HSPs), including heme oxygenase-1 (HO-1; also known as HSP32), HSP60 and HSP70;
- the thioredoxin (Trx)/thioredoxin reductase (TrxR) system;
- Sirtuins.

Later, SOD, the main antioxidant enzyme of the first line of antioxidant defence in the cell, was also included into the vitagene family (Surai, 2016; Surai and Fisinin, 2016c). In general, the products of the aforementioned genes are involved in controlling diverse forms of cellular stress, regulate redox balance and various signalling pathways leading to adaptation to stress. The protective roles of HSPs, thioredoxin systems, sirtuins and SOD as an important part of the vitagene network have been recently reviewed (Surai and Fisinin, 2016c). Furthermore, protective actions of HSPs (Surai, 2015a) and SOD (Surai, 2016) in poultry production were also analysed in detail. In addition, our analysis of recent publications clearly indicates that the vitagene network can be modulated by nutritional means (Surai et al, 2017a). In particular, vitamins A, D, E, C, as well as selenium, carnitine, betaine and silymarin are shown to affect vitagenes and improve adaptive ability of birds to various stresses. In fact, protective effects of the aforementioned nutrients on vitagenes are most pronounced in stress conditions. Indeed, these results are the first step to go from the development of the vitagene concept in avian/animal nutrition to designing commercial products which could help fight stresses in the commercial conditions of poultry/animal production. However, further work is required to understand the molecular mechanisms of the interactions between vitagenes and various signaling systems and

transcription factors in the cell to build adequate adaptive responses and minimize detrimental consequences of stresses in poultry and farm animals.

Among nutrients regulating vitagene network selenium has a special place. Recently, organic Se concept has been developed and successfully tested in poultry/animal production (Surai and Fisinin, 2016d; Surai et al., 2018). Indeed, Se efficacy depends on the dietary Se form used with products containing SeMet provide a natural form of Se to meet requirement of farm animals and poultry in stressful commercial conditions of meat, milk and egg production (Surai et al., 2018). When characterising antioxidant properties of Se, one has to take into account that in animals, including poultry, and humans Se in the form SeCys acts as an essential part of 25 selenoproteins which are located in different parts of the cell, expressed in different tissues and their expression depends on stress-conditions and Se availability. It seems likely that selenoproteins have a special protective role to play in the animal/chick gut. In particular, specific gastro-intestinal GSH-Px located in the intestine and responsible for decomposition of lipid peroxides. In fact, if activity of this enzyme is optimal the lipid peroxides found in the digestive tract will not be able to be absorbed and as a result will not be found in the blood. However, if the activity of this enzyme is low lipid peroxides can escape pre-absorptive detoxification leading to their incorporation into plasma lipoproteins. Furthermore, the possibility of participation of gut microbiota in nano-Se assimilation and metabolism (Surai et al., 2017c) needs further investigation and advantages and disadvantages of nano-Se as a source of Se in animal/poultry nutrition await further critical evaluations. Clearly healthy gut is one of the most important determinants of the general health of human and animals.

Future directions

In the last decade it has been proven that antioxidant-prooxidant (redox) balance in the gut (Surai and Fisinin, 2015) is closely linked to the vitagene network (Surai and Fisinin, 2016c) and gut microbiota is involved in regulation of the gut integrity, inflammation and health. It seems likely that changes in gut microbiota due to nutritional or environmental stresses could potentially affect gut redox potential and be responsible for enteritis (clinical and subclinical) and deterioration of FCR (Surai et al., 2017b). As yet, very little is known about relationship between gut microbiota, redox balance in the intestine and the vitagene network in the intestine and these questions await further investigation.

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Can Carbon Smart Agriculture Help the Livestock Industries Meet Rising World Food and Sustainability Demands?

L'agriculture intelligente face au climat peut-elle devenir une réponse des industries de l'élevage aux demandes mondiales accrues en matière de production alimentaire et de durabilité?

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Abstract

The rising world population brings with it the complex challenges of increasing food insecurity, a changing climate, increased demand for sustainable production and increasing animal welfare demands from a more demanding consumer. At the other extreme, livestock are integral to many rural livelihoods, being viewed as much more than just a food source particularly in developing countries. In contrast, the world's rising middle class is predicted to increase by 3.1B people by 2030, with increased demand for animal-based protein in this demographic. The net effect is that demand for livestock products in developed countries is likely to decline, demand from subsistence agriculture is unlikely to change, but the rising world middle class provides an inexhaustible market for animal-based protein into the medium-term future.

Rising global awareness of the anthropogenic climate change has highlighted the relatively high environmental footprint of livestock production, in terms of water, nitrogen and greenhouse gas emissions. Coupled with animal welfare concerns, the social licence for the livestock industries to operate is now increasingly challenged, meaning that real action needs to be taken to secure the future place of these industries in many developed countries.

Carbon Farming and Climate Smart Agriculture have emerged as responses to reconcile these often-competing demands on our food system, focusing on the key principles required to adapt agriculture to a changing climate, while also reconciling apparent conflicts between productivity and sustainability. The future of the livestock industry is therefore one of valuable opportunities but also significant threats – neither can be ignored as we move into a future world, with more demanding consumers, increased food demand, but also increasing accountability for environmental impacts, sustainable production in a changing climate.

Résumé

Avec l'augmentation de la population sur la planète viennent les défis complexes de la sécurité alimentaire. D'un côté, on s'inquiète de plus en plus du bien-être des animaux et de l'empreinte écologique des productions animales et, parallèlement, on assiste à une tendance au végétarisme. À l'autre bout du spectre, le bétail fait partie intégrante du mode de vie en milieu rural dans beaucoup de pays en développement, où il est considéré nettement plus qu'une simple source de

nourriture. Et pourtant, on prévoit que la classe moyenne devrait augmenter de 3,1 milliards d'individus dans le monde d'ici 2030 et que cette tranche de la population fera croître la demande pour la protéine d'origine animale. L'effet net de ces phénomènes est que la demande pour les produits animaux dans les pays développés devrait fléchir, tandis que celle issue de l'agriculture de subsistance ne devrait pas changer, mais que la classe moyenne émergente à travers le monde offre un marché insatiable pour la protéine d'origine animale à moyen terme.

La prise de conscience grandissante à l'échelle planétaire concernant le changement climatique d'origine humaine a mis en lumière l'empreinte écologique relativement profonde laissée par les productions animales en ce qui concerne l'eau, l'azote, la déforestation et les émissions de gaz à effet de serre. Considérant en outre les préoccupations à l'endroit du bien-être animal, l'acceptation sociale des industries de l'élevage est de plus en plus contestée, de sorte que des gestes concrets doivent être posés pour assurer l'avenir de ces industries dans de nombreux pays développés.

Les pratiques agricoles qui favorisent le piégeage du carbone et l'agriculture intelligente face au climat sont autant de réponses visant à concilier ces pressions souvent contraires exercées sur notre système de production alimentaire et qui mettent en valeur les principes clés requis pour adapter l'agriculture au contexte du changement climatique, tout en cherchant à aplanir les apparentes contradictions entre la productivité et la durabilité. Cet article aborde les enjeux conflictuels auxquels les industries de l'élevage seront confrontées à l'avenir, compte tenu des principes de l'agriculture intelligente face au climat.

Introduction

The global population is predicted to reach 9.8 billion by 2050, after which the rate of growth is predicted to slow, resulting in estimates of a world population between 11.2 billion (mid scenario) through to about 14 billion (high scenario) by 2100 (United Nations 2017). This increasing world population, coupled with rising incomes of the world middle class, will increase food demand by between 60 to 70% by mid century, based on 2005/2007 production levels (Alexandratos and Bruinsma 2012).

The rising world population brings with it the complex challenges of food security. Food security exists when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life (FAO 2010) - noting that the definition includes those who have too much, too little and the wrong types of unhealthy food.

The majority of the added world population will need to produce their own food through subsistence agriculture and thus face the daily challenge of food security. In contrast, the world rising middle-class, estimated at 3.2 billion in 2016, rising to an estimated 4.9 billion by 2030 (Kharas 2017), will be demanding more and higher quality food. There is also a strong positive relationship between GDP per capita and the demand for animal protein (WHO 2003; Thornton 2010). This provides a potentially inexhaustible future market for countries producing and exporting livestock products.

Social licence to operate

Concerns over animal welfare and the environmental footprint of livestock production are now regularly the subject of media attention in developed countries; although notably receiving much less attention in developing countries, perhaps because of their closer connection to their agricultural sectors. Although driven by a small but vocal number of lobby groups, there is now a clear trend towards vegetarianism and veganism, mainly in younger people in more affluent countries. Coupled with lower rates of population growth and health messages of moderation in meat consumption, the net result appears to be a stagnation of consumption of livestock products per capita in the developed world (Figure 1), while consumption appears to be increasing rapidly in the developing world (Thornton 2010).

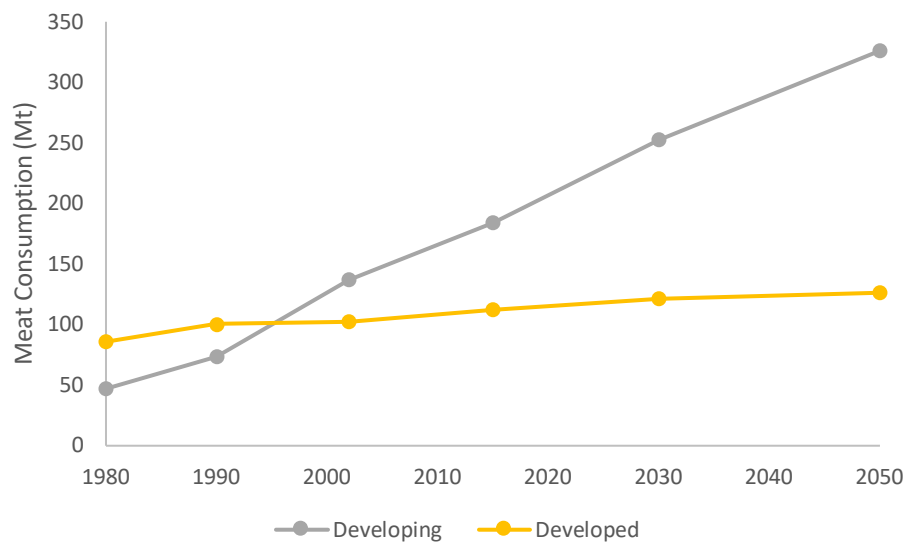


Figure 1. Past and projected meat consumption in developing and developed countries (after Thornton 2010).

However, of greater concern than simply declining rates of consumption, is the risk to the social licence for the livestock industries to operate in developed countries. Historically, strategic plans for the livestock industries focused on potential (positive) productivity gains from investment in research and industry development. Over the last decade, these strategic plans now include the risk or penalty to the industry (dollars below the baseline) of not meeting public expectations on animal welfare (e.g. slaughter practice and live exports) and environmental impacts (e.g. greenhouse gas emissions), with recent estimates of financial penalties now far greater than potential future productivity gains. Two clear examples would be Australia's Meat Industry Strategic Plan (note the large negative bar under Consumer and Community Support in Figure 2) and Canada's National Beef Strategy (e.g. CNBS 2015; MISP 2016).

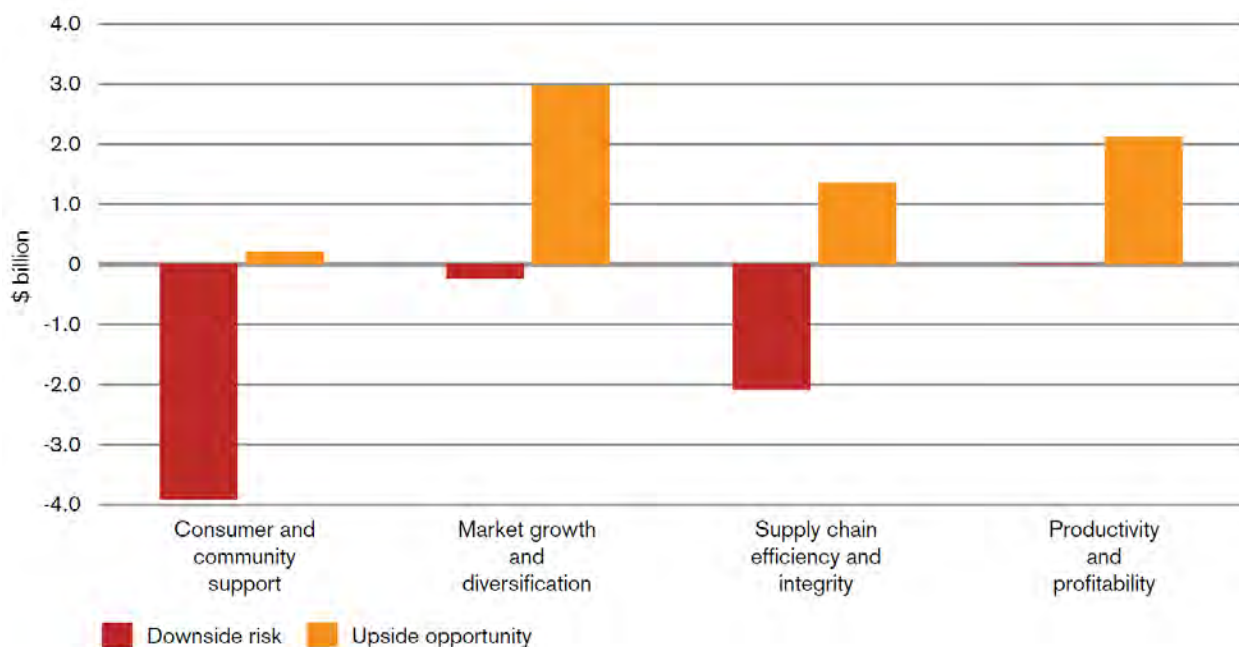


Figure 2. Forecast value of risks and opportunities to the Australian red meat industry to 2030, relative to the baseline net industry income increase of \$48,320 million by 2030 (in 2015 dollars and 5% real rate of return) (MISP 2016).

The livestock industries in developed countries are therefore coming under far more pressure and scrutiny than production systems in the developing world. While many of the environmental impacts could and should be addressed through research, there may well be a widening gap between community welfare expectations and best practice in the industry (i.e. animals will still need to be slaughtered, regardless of how humane this could ever be). This requires a coordinated program of research to understand first what the future target consumers want in terms of livestock products, including their welfare and environmental attributes, before working back to the livestock production systems of the future that can deliver these products.

Rural livelihoods

Livestock play a vital role in the economies of rural communities, not only in the developing world, but also in more extensive rangeland regions of the developed world. In many countries livestock are not only as a source of food, but are integral to religious rites, used as a measure of wealth and social status, used as a source of power in tilling soils, plus their waste being a vital source of nutrients for crop production (Thornton 2010; Henry *et al.* 2012). In 2006, the global livestock sector employed an estimated 1.3 billion people in the supply chain and directly supported the livelihoods of 600 million smallholder farmers in the developing world (Thornton *et al.* 2006). Livestock products contribute 17% to kilocalorie consumption and 33% to protein consumption globally, but with large and widening differences between rich and poor countries (Rosegrant 2009). Livestock are also the only practical means of converting grassland into food, where many of these rangeland systems are either not suitable for cropping.

In contrast, the world's rising middle class is predicted to increase by 3.1B people by 2030, with increased demand for animal-based protein in this demographic (Kharas 2017). The net effect is that demand for livestock products in developed countries is likely to continue to decline, livestock production from subsistence agriculture is likely to remain vital for their livelihoods and thus continue to increase in proportion to population growth, but demand from the rising world middle class will mean that net demand for livestock products globally will rise rapidly.

Climate Change

It is now well established that livestock production systems are both vulnerable to, but also contribute significantly to anthropogenic climate change (Steinfeld *et al.* 2006; Smith *et al.* 2007; Henry *et al.* 2012). Estimates of the livestock sectors contribution to global greenhouse gas emissions vary between 14 and 18 percent (Figure 3) (Steinfeld *et al.* 2006; IPCC 2014), sourced mainly from enteric methane and, to a less extent, nitrous oxide (Eckard *et al.* 2010). Higher figures than this have been published, varying from full life-cycle assessments that attribute pre-farm emissions, transport and land clearing to the livestock sector, through to the mathematically impossible >50% of global emissions largely used by lobbyists (Wedderburn-Bisshop *et al.* 2015).

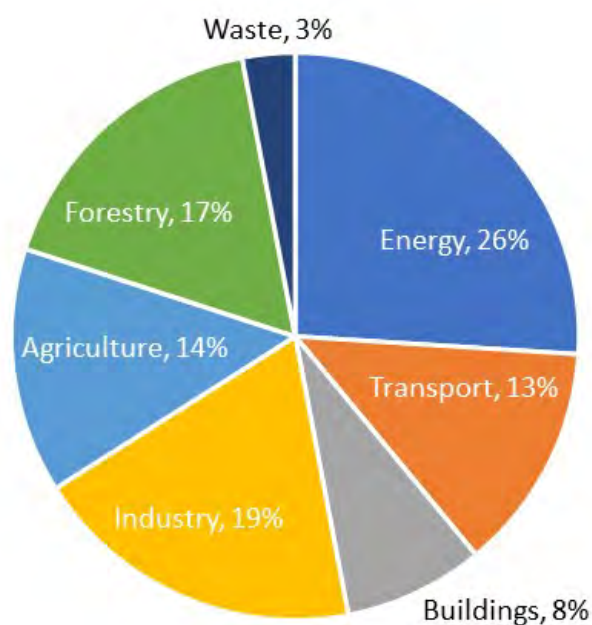


Figure 3. Greenhouse gas emissions attributed to sectors of the global economy (IPCC 2014).

With growing awareness of the likely negative impacts of climate change, there is now a clear trend away from fossil fuels through to renewable energy in both the energy and transport sectors. It therefore becomes an imperative for the agricultural sector as a whole to develop lower greenhouse gas emissions production systems, to avoid being the only remaining large emitting sector. While carbon offsets may be able to mitigate the net emissions of livestock

production in the short term, ultimately research to develop lower methane emitting ruminants is an unavoidable imperative for the future of the industry.

Livestock production systems will also be affected by a changing climate, both directly through increased heat stress, but also indirectly through impacts on pasture and forage production, feed availability, animal health, growth and reproduction, disease and pest distribution, increasing competition for land and even through changing market perceptions (Eckard 2012; Henry *et al.* 2012). These impacts can be positive in regions where low temperatures have historically limited forage growth (e.g. northern Canada, southern New Zealand), or even in regions where winter pasture growth is predicted to increase (Cullen *et al.* 2009), but may be quite negative in regions where temperature are already high and predicted to increase (e.g. northern Australia) (Howden *et al.* 1999). Clearly research is required to understand these regional impacts and provide cost effective adaptation options for livestock producers to adapt to these changes, including identifying new regions that may improve in their suitability.

Nitrogen

Livestock production is reported to be the largest anthropogenic influence on the global nitrogen cycle (Bouwman *et al.* 2013). Ruminants are also not particularly efficient at utilising dietary nitrogen, with cattle fed to requirement typically excreting 75 to 80% of the N they consume (Whitehead 1995), with only 30 to 40% of recycled N ultimately being available for plant growth (Ball and Ryden 1984; Whitehead 1995). The production of red-meat requires 84g of N per 1,000 kcal, compared to 16g of N for plant-based foods (Liu *et al.* 2016). Increasing N inputs into intensive grazing systems has also been shown to increase nitrate contamination of water supplies and groundwater (Eckard *et al.* 2004a; Eckard *et al.* 2004b) and increase emissions of ammonia and the greenhouse gas nitrous oxide into the atmosphere (Eckard *et al.* 2010). In contrast, extensive and subsistence livestock production systems are commonly N limited, with significantly lower N losses and environmental impacts, largely due to the effective dilution of N losses over large areas and further dilution from more diffuse land use.

While most developed countries have now imposed limits on N use in agriculture, there are also calls for a shift in human diets, with poultry or pork replacing red-meat to reduce N pollution (Bouwman *et al.* 2013). Further research is required to improve the overall N use efficiency of livestock production systems; while livestock production may never be as N use efficient as cropping systems by definition, there is clearly room for improvement. Further improvements in N use efficiency in livestock production systems is therefore an imperative, not only economically, but for the future sustainability of the industry.

Carbon Farming and Climate Smart Agriculture

Carbon Farming (CF) could be defined as being management principles that minimise greenhouse gas emissions (including N losses), maximise carbon sequestration in the landscape, while improving the productivity and resilience of agricultural systems. Climate-smart agriculture (CSA) is defined in much the same way, but perhaps being more a framework to facilitate reconciliation of these competing demands on agriculture (Lipper *et al.* 2014). The principles underpinning CSA/CF are not new and are reasonably well understood by science and increasingly by industry and producers. Perhaps the only missing component from CSA/CF, as defined above, would be consideration of animal welfare demands of future consumers. The key point of difference with CSA/CF is the focus on higher resilience and lower risks to productivity, whereas it could be argued a ‘business-as-usual’ approach ultimately leads to higher risks and possibly lower resilience.

The principles of CSA/CF would therefore assist in medium to long-term planning for livestock production systems through, for example, better matching future climate scenarios, land use capability and production systems. This may lead to the sustainable intensification of production in regions and catchments where climate variability is low (i.e. a predictable climate) or land use is more diffuse, but deliberately de-intensifying production in areas where future climate predictions show increased variability. For the livestock industries, this may create challenges and new opportunities. For example, areas where cropping is becoming less viable, perhaps through a combination of climate change and loss of soil carbon, the introduction of livestock into lower intensity mixed farming systems can confer significantly greater resilience to the farm. Clearly these core principles need to be adopted along the full value-chain, including policy and retailers, to ensure that farmers not just sent a ‘more-is-better’ signal, but are also rewarded for more sustainable and resilient production systems.

Conclusion

Demand for livestock products is likely to increase substantially into the future. However, for livestock producers in developed countries to continue to capture this benefit, it will require a significant and coordinated effort from farmers, through the supply chain, policy and research all working together to reconcile the competing challenges in a far more integrated way than in the past.

The future of the livestock industry is therefore one of valuable opportunities but also significant threats – neither can be ignored as we move into a future world, with more demanding consumers, increased demand for food, but also increasing accountability for environmental impacts and sustainable production in a changing climate. Carbon smart agriculture can therefore help the livestock industries meet rising world food demands and sustainability demands, through providing key principles to guide future research, development, policy and strategic planning for the livestock industries.

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High Inclusion Levels of Canola Meal in Broiler Chicken Nutrition

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Abstract

Solvent-extracted canola meal (CM) is commonly used source of protein for poultry. Dietary inclusion level of CM, however, has been historically limited to 5-10% due to concerns related to high fiber content and the presence of glucosinolates. A four-week feeding trial was conducted to determine the effect of varying inclusion levels of CM on the growth performance of broiler chickens. The experiment had four phases of six treatment groups: pre-starter (0, 3, 6, 9, 12, 15 % of CM), starter (0, 4, 8, 12, 14, 18 % of CM), grower 1 (0, 5, 10, 15, 20, 25 % of CM) and grower 2 (0, 6, 12, 18, 24, 30 % of CM), each lasting for one week. Diets were balanced for SID AA contents by replacing soybean meal (SBM) in a corn-SBM basal diet with graded levels of CM. One-day-old broiler chickens (housed 5 birds/cage) were allotted into the six treatment groups with ten replicates/cages per treatment. Body weight gain (BWG) and feed intake (FI) of birds were monitored weekly and feed conversion ratio (FCR) was calculated. Completely randomized design using the GLM procedure of SAS was used. Overall, the result showed that although NDF content of diets differed substantially with increased levels of CM bird performance was not significantly ($P>0.05$) affected by CM inclusion levels. Irrespective of the phase and CM inclusion levels, FI, BWG, and FCR averaged 2,019 g/bird/28 d, 1,387 g/bird/28 d, and 1.46 g/feed/g gain and were similar to 1,921 g/bird/28 d, 1,325 g/bird/28 d, and 1.45 g feed/g gain for the control treatment, respectively. Incremental levels of CM inclusion didn't have a significant ($P>0.05$) effect on NDF digestibility. It could be concluded that CM can effectively replace SBM when used up to 30% in broiler chicken diets assuming the diets are formulated on a digestible AA basis. Canola fiber has minimal effect on nutrient utilization as seen from the excellent performance of broiler chickens fed diets of different fiber content.

Key words: canola meal, inclusion level, broiler chicken, digestible amino acid, growth performance.

Weight and Ash Content of Whole and Parts of Tibia in Lohmann LSL-lite Hens Fed Different Levels of Calcium and 25-hydroxy vitamin D3 from 74 to 81 Week of Age

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Abstract

Skeletal calcium (Ca) resorption over the course of lay cycle can lead to progressive structural loss leading to osteoporosis, compromised welfare and death. We investigated the effect of feeding different levels of Ca and 25-hydroxy vitamin D₃ (25OHD₃) on the dried weight (DW) and ash content (AC) of whole and parts of tibia in Lohmann LSL-lite hens from 74 to 81 wk of age. Four levels of Ca (3.0, 3.5, 4.0 and 4.5%) and three levels of 25OHD₃ (0, 69 and 138 µg/kg) were tested in 84 individually caged hens. All diets contained 330 KIU of vitamin D₃/kg. Birds were necropsied at the start (baseline) and on 81 wk for tibia samples. Tibia ends (epiphysis and metaphysis) were separated from diaphysis. Diaphysis was cut longitudinally the medullary bone removed by scrapping and the remainder designated cortical. The ends, cortical and medullary sections were dried at 105°C, weighed and subsequently ashed at 600°C. The DW and AC of the whole and parts of tibia were reported as a change index (CI) calculated by dividing wk 81 values by baseline values and multiplied by 100. There was no ($P > 0.05$) interaction between Ca and 25OHD₃ in all measured variables. Neither Ca nor 25OHD₃ affected CI for whole tibia and ends DW. However, Ca linearly ($P < 0.01$) increased CI of medullary DW and AC and linearly decreased ($P < 0.01$) CI of whole tibia and ends AC. Feeding 25OHD₃ linearly ($P < 0.01$) increased CI of medullary DM but linearly ($P < 0.01$) decreased CI of cortical DM. For the CI of AC, 25OHD₃ linearly ($P < 0.01$) increased medullary value and concomitantly reduced ($P < 0.05$) the values for whole tibia and cortical in linear fashion. In conclusion, feeding hens Ca and 25OHD₃ from week 74 to 81 of age independently improved AC in medullary bone at the expense of other parts of the tibia. These findings suggest hens at this age have higher propensity to maintain medullary bone and dietary interventions such as increased offerings of Ca over 4.0 % and 25OHD₃ more than 69 µg/kg may have adverse effects on structure of ends and cortical bones at this age, respectively. However, supplementation of 69 µg/kg 25OHD₃ increased medullary AC, while it did not negatively affect cortical AC.

Keywords: calcium, vitamin D, bone, medullary, cortical, ash, ageing

Hammermilling of Soft and Hard Durum Wheats Create Starch Damage which Improves AME and Starch Digestion in Broiler Chickens

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Abstract

A 2×2 factorial experiment was used to study the impact of durum wheat hardness (soft vs, hard) and fineness of hammer mill grinding (screen hole size- 2.4 vs 3.6mm) on starch damage level and its impact on starch digestibility in broiler chickens. A total of 125-day-old Ross 308 chicks were allocated to 1 of 4 treatments, which were replicated 5 times with five broiler chickens per replicate (cage). Hammer mill resulted in starch damage in both wheat types, however, it was significantly lower ($P = 0.0001$) in soft wheat at both fine and medium grind than in hard durum wheat; 2.12 and 1.92 vs 3.94 and 3.60 (% of total starch) for soft wheat fine grind, soft wheat medium grind, hard wheat fine grind and hard wheat medium grind respectively. Average of particle size was smaller ($P = 0.0001$) for soft wheat than hard wheat in both hole sizes of grinding and water absorption was higher ($P = 0.0001$) with medium grind compared to fine grind in both wheat types. Scanning Electron Microscope morphology analysis revealed that starch granules in soft wheat had smooth and flat edges whereas hard wheat had irregular and more fractal dimensions due to the mechanical damage of starch. Grind size had no effect on feed intake, body weight or feed efficiency ($P < 0.05$). Treatment had no effect on organ weights (% of BW) except the empty crop which was larger ($P = 0.004$) for birds fed coarse ground material and may reflect an increased feed retention time. Total tract starch digestibility of hard wheat was higher ($P = 0.02$) than soft wheat (97.89 vs 97.07 %). Likewise, AME of hard durum wheat was higher than soft 2944, 3000 vs 2807, 2508 kcal/kg, fine grind hard wheat, medium grind hard wheat, fine grind soft wheat, medium grind soft wheat, respectively. Starch damage was positively correlated with AME ($P = 0.0005$) while particle size had no effect. In conclusion, grind size and wheat type affected level of starch damage, energy utilization and starch digestion, however, the effect of grind size on these parameters was more pronounced in soft than hard wheat.

Key words: soft and hard wheat, starch, morphology, AME, broiler.

Predicted Production and Bioavailability of Volatile Fatty Acids in the Hindgut of Growing Pigs: Impact of the Interaction of Dietary Fiber and Lipid Types

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Abstract

In the recent years, the use of cereal co-products has significantly increased in pig diets resulting in increased dietary fibre (DF) content. Feed compounders fortify high-fibre diets with lipids to meet the energy content of a balanced diet, however, interactive effects of DF and lipid types has not been systematically studied in swine. A combination of *in vivo* (ileal and caecal cannulated pigs) and *in vitro* (caecal or faecal inoculum-based fermentation) methodologies were used to investigate the interactive effects of dietary fiber and lipid types on production and absorption of volatile fatty acids (VFA), and fermentability of organic matter (OM) in hindgut segments. Eight ileal- and caecal-cannulated Yorkshire barrows were fed either pectin- or cellulose-containing diets that were supplemented with either corn oil or beef tallow. Pigs were allocated diets in two independent Youden Latin squares with a 2×2 factorial arrangement of treatments. Ileal and caecal digesta were collected, freeze-dried and fermented using inoculum from fresh caecal digesta and faeces, respectively, to predict VFA production and bioavailability, and fermentability of OM. There were interactions ($P < 0.05$) between DF and lipid type on the determined and predicted quantity of VFA produced and absorbed in the caecum and colon. In this context, the predicted amounts of VFA produced and absorbed in the caecum or colon were higher ($P < 0.05$) for pectin diets compared to cellulose diets. Supplementation with beef tallow depressed the determined ileal and predicted caecal fermentability of OM and acetic and propionic acids production ($P < 0.001$) in the colon more in pigs fed pectin diets compared to those fed cellulose diets. In conclusion, the predicted quantity of VFA produced and absorbed, and fermentability of OM in the caecum and colon is greater in pigs fed pectin diets compared with those fed on cellulose diets, and beef tallow supplementation depressed fermentation. The take home message is that if the benefits of fermentation are to be achieved, nutritionists should give a careful consideration when supplementing pig high-fiber diets with animal lipid sources. Gastrointestinal concentrations of VFA could be used as reliable estimates for unabsorbed VFA rather than VFA production.

Keywords: absorption; double cannulated pigs; insoluble fiber; *in vitro* fermentation; short-chain fatty acids; soluble fiber.

Egg production and Quality Responses to Increasing Isoleucine Supplementation in a Low Crude Protein Corn-Soybean Diet Fed to Pullets 19 to 48 Weeks of Age

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Abstract

The economic and social challenges egg producers face today can largely be attributed to high feed costs alongside emerging trends in housing and production practices. Reducing dietary protein could be a means of alleviating these challenges; however, significant protein restriction may negatively affect performance. Formulating diets on an ideal protein basis allows for reductions in crude protein (CP) without hindering performance, given all limiting amino acids are supplied at the required levels. Isoleucine (Ile) has been speculated to be potentially limiting in low CP corn-soybean diets, however supporting data is limited. Therefore, the objective of this study was to identify the ideal level of Ile in a diet reduced by 2% CP (LCP) for optimal laying performance in pullets during phase 1 (19-27 w/o) and 2 (28-48 w/o) of production. One hundred and eighty Shaver hens were provided either a standard CP (18 and 16% for phases 1 and 2, respectively) control diet (80% SID Ile:Lys) or a LCP diet supplemented with increasing levels of Ile (70, 80, 90 and 100% SID Ile:Lys). Feed intake, body weight, egg production, egg quality (weight, composition, Haugh unit [HU], and shell strength) and serum uric acid were affected ($P < 0.05$) by diets. Hens fed 100% Ile:Lys exhibited the highest body weight and lowest egg weight, whereas the reverse is true for hens fed the control and 70% Ile:Lys diet. A period*diet interaction was seen for egg production, suggesting that Ile requirements may change as hens age. Egg quality results suggest that additional Ile supports the production of albumin protein, as indicated by greater % albumin and HU for the 90 and 100% Ile:Lys diets. Serum uric acid exhibited a quadratic response to Ile, with the lowest levels in hens fed the 90% Ile:Lys diet. The current data suggests that the ideal level of Ile for optimal egg production and quality responses is between 70-90% SID Ile:Lys for hens fed LCP diets.

Keywords: isoleucine, LCP, egg production

Nutrient Profile of the PMR May Affect Rumen Fermentation more than the Type of Pellet Fed

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Abstract

Feeding management in automated milking systems (AMS) consists of a partial mixed ration (PMR) and concentrate provided in the AMS, with the goal of encouraging visits to the AMS while meeting nutrient requirements. Several studies have focused on the effect of amount and type of concentrate fed; however, little research has evaluated how nutrient allocation between the PMR and pellet can affect feed intake and rumen fermentation when the same overall diet is provided. An experimental diet was formulated for total DMI of 25 kg/d and a portion removed as either a fibre (F; 7.7% starch, 41.2% NDF) or starch (S; 59.5% starch, 14.7% NDF) pellet, fed at either 3 kg (H) or 1 kg (L) twice per day. For all pellet treatments, four complementary PMR were formulated such that the overall diet (PMR + pellet) was the same among all treatments. Eight ruminally cannulated cows were used in a 4 × 4 Latin square design study with 14-d periods, with treatments of FH, FL, SH, and SL. Cows were fed PMR once daily at 1200 h, and pellet twice daily at 0600 and 1800 h. Disappearance of PMR was measured in 3 h increments following PMR delivery, and rumen pH was measured for a 72-h period. Although there was no difference in total DMI among treatments (27.7 kg/d), PMR intake was higher (25.3 vs. 22.9 kg/d; $P < 0.01$) and severity of acidosis tended to be greater (42.3 vs. 25.6 pH × min/d; $P = 0.06$) when less pellet was fed, regardless of type. In addition, contrary to our hypothesis, the F pellet treatment tended to increase duration of acidosis (210 vs. 103 min/d; $P = 0.08$), which may be attributed to slug feeding; cows consumed more high-starch PMR immediately after delivery (33.5 vs. 28.5 % of PMR intake; $P = 0.04$) compared to the S pellet treatment where intake of the high-fibre PMR was distributed throughout the day. These results indicate that intake and nutrient profile of PMR may have a greater influence on rumen fermentation than the pellet, and this should be considered when formulating diets where PMR is fed.

Key words: partial mixed ration, rumen fermentation

Supplementation of a *Saccharomyces cerevisiae* Fermentation Product during the Calving Transition Period may Decrease Inflammation of Dairy Cows

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Abstract

Cows experience a depressed immune system during the transition period, and it is important to feed a diet that can enhance the immune response. Therefore, the objective of this study was to measure indicators of oxidative stress and humoral immunity and the acute phase response of transition dairy cows fed a *Saccharomyces cerevisiae* fermentation product (SCFP, NutriTek®, Diamond V, Cedar Rapids, IA). Twenty-eight days before expected calving date, cows ($n = 38$) were fed a controlled-energy close-up diet with 13% starch with (SCFP) or without (CON) SCFP. After calving cows were fed high or low starch (HS vs. LS; 27 and 21%, respectively) diets with or without SCFP for 3 weeks. Animals were assigned to one of four treatments (CON+HS, CON+LS, SCFP+HS, SCFP+LS) in a randomized block design. On d 7 and 21 after calving, cows underwent ovalbumin challenges to measure humoral immune response, but it was not affected by SCFP or dietary starch content treatment. Although postpartum dietary starch content had no effect on serum concentrations of acute phase proteins or indicators of oxidative stress, the SCFP treatment decreased serum concentration of haptoglobin on d 7 after calving (0.26 vs. 0.62 mg/mL; $P = 0.03$), suggesting that SCFP decreased inflammation. However, SCFP increased plasma malondialdehyde concentrations for primiparous cows fed the HS diets (20.8 vs. 15.2 μ M; $P = 0.03$), and decreased total-antioxidant capacity of plasma for multiparous cows fed the LS diets (0.57 vs. 1.05 mM; $P = 0.03$), indicating that SCFP failed to alleviate oxidative stress. Supplementation of SCFP did not affect humoral immunity nor reduce oxidative stress, but decreased serum haptoglobin concentration after calving, which suggests that the extent of postpartum inflammation may be reduced by feeding a *Saccharomyces cerevisiae* fermentation product.

Key words: humoral immunity, acute phase response, *Saccharomyces cerevisiae* fermentation product, oxidative stress

The Effect of Fibrolytic Enzymes on Lactation Performance, Feeding Behavior, and Digestibility in High Producing Dairy Cows Fed a Barley Silage-Based Diet

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Abstract

The objectives of this study were to evaluate the effects of pre-treating dairy cow rations with a fibrolytic enzyme, *Trichoderma reesei*-derived fermentation extract (FETR, mixture of xylanase and cellulase; AB Vista, UK) on lactation performance, digestibility, and feeding behavior of dairy cows fed a barley silage-based diet. Eight Holstein dairy cows were assigned randomly to one of four treatments: 0, 0.5, 0.75, and 1 ml of FETR/kg DM of diet in a replicated Latin square design. The pre-treatment was applied to the complete diet during the mixing process. The milk fat yield, fat corrected milk and the energy corrected milk quadratically responded to the incremental levels of FETR. The milk protein percentage linearly improved in response to FETR. Increasing FETR levels resulted in a quadratic effect on feed efficiency. There was no effect of FETR level on feeding behavior. In conclusion, pre-treating dairy cow barley silage-based diet with FETR at the optimal dosage (0.75 ml FETR/kg DM) increased the feed efficiency without affecting DMI. The positive effect of adding FETR could benefit the dairy industry in Western Canada where barley silage-based diets are common.

The Effect of Calcium Oxide Treatment of Barley Straw on In Vitro Digestibility

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Abstract

Improving the nutritional value of low quality forages is one strategy to lower feed costs for beef cattle. The objective of this experiment was to determine the extent to which calcium oxide (CaO) treatment can improve the digestibility of barley straw, as compared to untreated barley straw, barley silage and corn silage. Barley straw was hydrated to 50% moisture and mixed with CaO at 5% of straw dry matter. *In vitro* degradation of CaO-treated straw, untreated straw, corn silage and barley silage was measured using the Ankom DAISY^{II} Incubator system. Inoculum was collected from 4 ruminally cannulated heifers fed a wheat-based finishing diet with either barley silage or untreated barley straw as the forage source (2 animals per diet). Inoculum from each diet was combined and used to incubate forage samples for 30 h. CaO was found to reduce the NDF content of barley straw by 15.1% (75.6 vs. 64.2%). CaO did not further improve the digestibility of the remaining residual NDF over that of untreated straw (31.4% vs. 31.1%, $p=0.99$). CaO treatment improved true DM digestibility (TDMD) of barley straw from 48.4 % to 55.8% ($p<0.01$), but was still lower than the TDMD of barley (65.4%) and corn silage (64.8%). Forages incubated in inoculum collected from heifers fed straw tended to have higher NDFD ($p=0.07$) and TDMD ($p=0.06$) than those fed barley silage. In conclusion, treatment of barley straw with CaO improved TDMD as compared to untreated barley straw. No effect was observed on NDFD with CaO treatment, likely as a result of the reduction in NDF content of the original substrate. Source of rumen inoculum impacted the NDFD and TDMD of all forages, possibly due to greater activity of fibrolytic bacterial species in heifers fed the diet that included barley straw.

Keywords: calcium oxide, straw, digestibility, beef cattle

Effects of Different Forms and Concentrations of Iron on Growth in Wild-Type and Iron-Uptake Defective Mutants of *Salmonella*

Zhigang Tan ^{1,2}, Hai Yu², Samuel Chekabab², Moussa Diarra², Joshua Gong², and Chengbo Yang¹

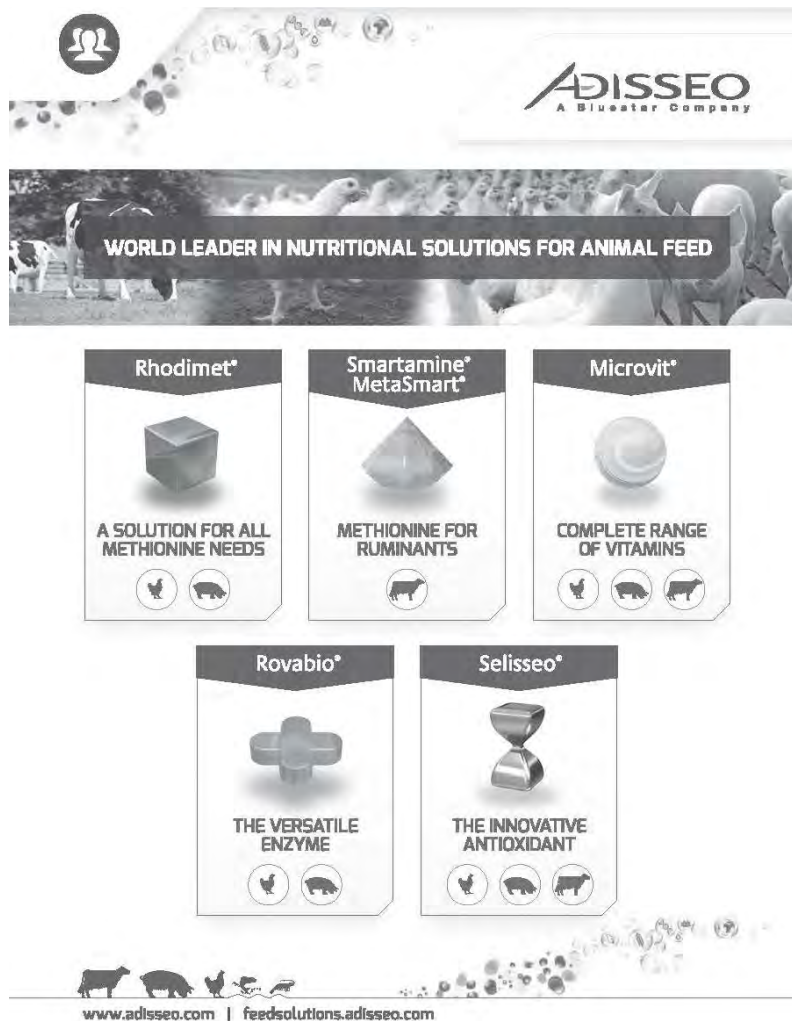
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Abstract

















Salmonella species are one of the hazardous pathogens causing contamination of feed ingredients and posing a critical threat to human and animal health. Iron acquisition is essential for *Salmonella* colonization and biofilm formation. However, there are no reports on the effects of different iron forms and iron-uptake systems on the growth of *Salmonella*. The present study was conducted to investigate the effects of different forms of iron, including ferric chloride, ferric citrate, ferric EDTA, ferrous-L-ascorbate and ferrous sulfate, on the growth of wide type and iron-uptake defective mutants of *Salmonella*. There were a total of six *Salmonella* strains used in this study, including *Salmonella enterica* Enteritidis 3346 (*S. Enteritidis* 3346), *Salmonella enterica* Typhimurium 3128 (*S. Typhimurium* 3128) and their iron-uptake defective mutant strains having ▲*TonB* and ▲*FepA*, *FhuA*, *iroN*, respectively. The iron concentrations of different iron forms were within a range of 0.1 - 50 µM. Results indicate that both wild-type and mutant *Salmonella* strains had better growth when treated with a higher dose of iron regardless the iron forms ($P < 0.001$) and iron-uptake defective mutants were detrimental to the growth of *Salmonella* ($P < 0.001$). These results suggest that reducing iron availability to *Salmonella* could be a promising approach to control *Salmonella* colonization. This study will provide industries with new nutritional strategies for the control of *Salmonella* to help strengthen animal health. Moreover, this study will help us to identify which iron-uptake protein is most essential for *Salmonella* colonization and biofilm formation and then to develop methods targeting the iron-uptake protein to reduce iron availability for *Salmonella*.

Keywords: Iron, Iron-uptake defective mutants, *Salmonella*



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Déjeuner du jeudi

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Pause-café du jeudi

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