Choline and Methionine for Transition Cows: Separating Fact from Fiction

Ric R. Grummer
Professor Emeritus
Department of Dairy Science, University of Wisconsin-Madison

Introduction

Research conducted on non-ruminant animals has clearly demonstrated an interrelationship between the nutrients choline and methionine, largely due to the common characteristic of them being methyl donors. In the field, there are many statements being made: e.g., choline can spare methionine, methionine can spare choline, if you feed methionine you don't need to feed choline, choline is a required nutrient for transition cows, choline is only needed for fat cows, and methionine can prevent fatty liver. These statements are largely based on research findings in non-ruminants. Is it correct to assume that these statements also hold true for transition dairy cows? The objective of this paper is to separate fact from fiction. That said, it is important to note that there is a paucity of data on the subject of choline-methionine interrelationships in ruminant animals.

Common Biology of Choline and Methionine

Dietary choline and methionine are extensively degraded in the rumen (Sharma and Erdman, 1988a), hence they must be fed in a form that minimizes ruminal degradation and maximizes flow to the small intestine. Both compounds contain methyl (**-CH_3**) groups which is the main basis for them being metabolically related. Choline is a constituent of phosphatidylcholine (**PC**) which is present in every cell membrane in the body and is a component of milk fat globule membranes. PC is also a component of lipoproteins that are responsible for transporting fat throughout the body. As a constituent of very low density lipoproteins (**VLDL**), PC is required for fat export out of the liver. Fatty liver is the classic deficiency symptom for choline deficiency, and the development of fatty liver in 50% of transition cows has been attributed to the lack of absorption of dietary choline during the transition period (Grummer, 2012).

Cows can synthesize PC endogenously, and clearly there is sufficient endogenous synthesis except during the transition period when fatty acid mobilization from adipose tissue is great and fatty acid uptake by the liver increases dramatically. Endogenous synthesis of PC occurs by methylation of phosphatidylethanolamine (**Figure 1**). The methyl groups for this can be derived from methionine. Hence the close metabolic relationship of the two compounds and the observation in non-ruminants that methionine can spare choline and choline can spare methionine.

[1] Contact: E-Mail: rgrummer@wisc.edu

One of the exciting recent discoveries is that gene expression can be regulated by DNA methylation. Therefore, choline and methionine can potentially be involved in regulation of an infinite number of metabolic pathways. This area of investigation is in its infancy.

Compared to non-ruminants, very little is known about choline-methionine relationships. A classic study conducted by Emmanuel and Kennelly (1984) in lactating goats indicated that 28% of methionine was utilized for choline synthesis and 6% of the choline pool was derived from methionine. Interestingly, choline methyl groups were not used for synthesis of methionine. Sharma and Erdman (1988b) obtained greater milk production responses in dairy cattle to postruminal infusion of choline vs. methionine in the presence of a methylation inhibitor suggesting that methionine methyl groups can be used for the synthesis of choline.

Effects of Choline and Methionine on Fatty Liver

During the transition period, due to fatty acid mobilization, fatty acid uptake by the liver increases from 100 to approximately 1300 g/day (Overton, unpublished). If there is not sufficient PC to synthesize VLDL to export the fatty acids as triglyceride, fatty liver can result. Most (Cooke et al., 2007; Zom et al., 2011; Lima et al., 2012; Elek et al., 2013), but not all (Zahra et al., 2006; Zhou et al., 2016) studies indicate that feeding protected choline pre- and postpartum can reduce fat accumulation in the liver during periods of intense fatty acid mobilization. he same cannot be said for feeding protected methionine or methionine analogs. In six studies conducted thus far (Socha, 1994; Bertics et al., 1999; Piepenbrink et al., 2004; Preynat et al., 2010; Osario et al., 2013; Zhou et al., 2016), none have reported a reduction in liver fat due to methionine supplementation. Any claims that feeding protected methionine can replace feeding protected choline for prevention or treatment of fatty liver have not been substantiated. On a weight basis, choline has 4.3 times more methyl groups than methionine, therefore, it is possible that doses of methionine used in these studies were not sufficient enough to reduce fat accumulation in the liver. A second explanation may be that ruminants differ from non-ruminants in hepatic PC metabolism. More on this possibility below.

Effects of Choline and Methionine on Milk Production

A meta-analysis of thirteen studies (Grummer, 2012) in which protected choline supplementation had begun prepartum revealed increased postpartum dry matter intake (1.6 lb/day), milk yield (4.9 lb/day), fat yield (0.254 lb/day), and protein yield (0.167 lb/day). Termination of supplementation varied from calving day to 120 days postpartum, however, there was no difference in milk response for cows that were supplemented for less than thirty days postpartum versus those supplemented equal to or greater than thirty days postpartum. Interestingly, none of the studies monitored the performance of cows following supplementation. However, in a recent study (Zenobi et al., 2016) a carryover effect of feeding protected choline on milk production was observed following termination of supplementation.

A common misconception is that cows only respond to choline when diets are not "balanced" for methionine. This is clearly not true. In trials balanced for methionine (Piepenbrink and Overton, 2003; Ardalan et al., 2011; Lima et al., 2012; and Zenobi et al., 2016) the milk response has been consistent with the response derived from the meta- analysis.

A summary of trials monitoring production responses to feeding protected methionine or methionine analogs pre- and postpartum are in **Table 1** (Overton et al., 1996; Phillips et al., 2003; Piepenbrink et al., 2004; Ghorbani et al., 2007; Ordway et al., 2009; Preynat et al., 2009; Osorio et al., 2013; Zhou et al., 2016). Milk yield responses have been inconsistent. Increases in milk protein percentage have been the most consistent response seen. The most impressive responses have been in recent studies from the University of Illinois (Osorio et al., 2013; Zhou et al., 2016) in which supplemented diets have been formulated to contain metabolizable lysine:methionine ratios below 3.

Effects of Choline and Methionine on Reproduction

Several studies have observed large increases in first service conception rates when feeding protected choline (Oelrichs et al., 2004, 29 vs 58%; Shahsavari, 2012, 25 vs 40%; Zenobi et al., 2016, 24 vs 41%). However, these studies utilized few animal numbers (less than 50 per treatment) which limited statistical power. The Oelrichs study obtained a significant improvement and Zenobi study noted a tendency for improvement. Two larger studies on commercial farms observed either a nonsignificant numerical increase (Lima et al., 2012, 41 vs 48%; 165 cows per treatment) or a significant decrease (Amundson, 2014, 46 vs 40%; > 900 cows per treatment). The mechanism of action for an increase in conception rate is not known, but it may be related to a choline requirement for embryonic development.

Feeding protected methionine from calving to flushing altered gene expression in embryos; some of the changes were for genes related to embryo development and immune responses (Penagaricano et al., 2013). Embryos had greater lipid content when dams were fed protected methionine from three weeks prepartum to 30 days postpartum (Acosta et al., 2016). The researchers speculated that the improved energy status of embryos may facilitate superior embryo survival. Although first service conception rate was not affected, embryo loss following first service was reduced by feeding protected methionine from 31 to 127 days postpartum (0 vs 8.9% for control; Toledo et al., 2015). More studies are needed to evaluate the effects of supplementing methionine during the transition period on reproductive performance.

Head to Head Comparisons: Choline vs Methionine

There have been four studies that have utilized a factorial design (2 x 2; 4 treatments = control, methionine, choline, and methionine plus choline) to examine the effects of rumen protected choline and methionine on transition cows and to determine if there are any interactions between the two compounds. Ardalan et al. (2011) fed

treatments from 4 weeks prepartum to 10 weeks postpartum and observed increases in dry matter intake (3.0 and 6.9 lb/day for methionine and choline, respectively) but only choline increased milk yield (6.4 lb/day). Soltan et al. (2012) fed treatments from calving until 96 days postpartum and observed an increase in dry matter intake for choline which was greater (3.7 lb/day) when methionine was not fed than when it was fed (0.6 lb/day). Milk yield response to choline was also greater when methionine was not fed (4.2 vs 1.5 lb/day). Sun et al. (2016) did not observe interactions between feeding rumen-protected choline and methionine; choline increased dry matter intake, milk yield, and milk fat percentage while methionine increased dry matter intake, milk yield, and milk protein percentage. Finally, Zhou et al. (2016) observed no effects of choline but large effects of methionine on dry matter intake (4.6 lb/day), milk (8.8 lb/day), and milk protein percentage (0.18 units) when treatments were applied from 21 days prepartum until 30 days postpartum. The discrepancies between these studies are difficult to explain but may be related to differences in basal diets, amount and source of supplements, length of feeding, etc.

Wisconsin researchers (Chandler et al., 2015) have used liver cell cultures to study the effects of methionine and choline on metabolism. As expected, increasing concentrations of methionine in the media reduced expression of methionine synthase, an important gene controlling methionine formation. Choline had no effect. Interestingly, addition of methionine had no effect on expression of PEMT, an important gene in regulating methylation of phosphatidylethanolamine to form PC. This may be a reason why supplementing transition cows with methionine has not reduced fat accumulation in the liver. Consistent with this observation was that methionine did not enhance VLDL (i.e. fat) export from the cells (McCourt et al., 2015). These studies were the first to directly demonstrate that choline does enhance VLDL export from bovine liver cells which explains why supplementing rumen-protected choline to transition cows reduces fatty liver. Finally, oxidative stress of liver cells was reduced by choline but not by methionine.

Conclusions

Limited evidence does suggest that there are inter-relationships between choline and methionine in transition cows. Clearly, choline and methionine are both essential nutrients and both should be fed to transition cows in a rumen-protected form. Choline and methionine have unique roles and they can’t simply be substituted for one another in transition cow diets. For example, methionine increases milk protein percentage but choline apparently does not. Conversely, choline decreases liver fat but methionine, at levels tested, does not. Choline increases milk yield and methionine may as well, but initial evidence does not suggest that their effects are additive. Although more research is needed, there is sufficient evidence in the literature to clarify many of the misconceptions that are prevalent in the industry.

References

Acosta, D. A. V., A. C. Denicol, P. Tribulo, M. I. Rivelli, C. Skenandore, Z. Zhou, D. Luchini, M. N. Correa, P. J. Hansen, F. C. Cardoso. 2016. Effects of rumen-protected methionine and choline supplementation on the preimplantation embryo in Holstein cows. Therio. 85:1669-1679.

Amundson, M. C., P. D. Carvalho, R. W. Bender, R. R. Grummer, R. D. Shaver, and P. M. Fricke. 2014. Effect of rumen-protected choline top-dressed during the transition period on milk yield and composition in Holstein dairy cows on two commercial dairies. J. Dairy Sci. (E. Suppl. 1):244.

Ardalan, M., M. Dehghan-Banadaky, K. Rezayazdi, and N. Ghavi Hossein-Zadeh. 2011. The effect of rumen-protected methionine and choline on plasma metabolites of Holstein dairy cows. J. Agric. Sci. 149(5):639–646.

Bertics, S. J., and R. R. Grummer. 1999. Effects of fat and methionine hydroxy analog on prevention and alleviation of fatty liver induced by feed restriction. J. Dairy Sci. 82:2731-2736.

Chandler, T. L., C. L. McCourt, S. J. Bertics, B. A. Barton, and H. M. White. 2015. Methyl-donors choline and methionine differentially alter hepatic methyl carbon metabolism. J. Dairy Sci. 98 (Suppl. 2):132.

Cooke, R. F., N. Silva Del Rio, D. Z. Caraviello, S. J. Bertics, M. H. Ramos, and R. R. Grummer. 2007. Supplemental choline for prevention and alleviation of fatty liver in dairy cattle. J. Dairy Sci. 90:2413-2418.

Elek, P., T. Gaal, and F Husveth. 2013. Influence of rumen-protected choline on liver composition and blood variables indicating energy balance in periparturient dairy cows. Acta Vet. Hung. 61:59-70.

Emmanuel, B. and J. J. Kennelly. 1984. Kinetics of methionine and choline and their incorporation into plasma lipids and milk components in lactating goats. J. Dairy Sci. 67:1912-1918.

Ghorbani, G. R., D. Kianzad, M. Alikhani, A. Nikhah. 2007. Rumen-protected methionine improves early-lactation performance of dairy cattle under high ambient temperatures. Asian J. Anim. Vet. Adv. 2:184-195.

Grummer, R. R. 2012. Choline: A limiting nutrient for transition dairy cows. Proc. Cornell Nutr. Conf. Pre-symposium.

Lima, F.S., M.F. Sa Filho, L. F. Creco, and J. E. P. Santos. 2012. Effects of feeding rumen-protected choline on incidence of diseases and reproduction in dairy cows. Vet. J. 193:140-145.

McCourt, C. L., T. L. Chandler, S. J. Bertics, B. A. Barton, and H. M. White. 2015. Supplementation of choline increases very low density lipoprotein export from bovine primary hepatocytes. J. Dairy Sci. 98 (Suppl. 2):*ii*.

Oelrichs, W. A., M. C. Lucy, M. S. Kerley, and J. N. Spain. 2004. Feeding soybeans and rumen-protected choline to dairy cows during the periparturient period and early lactation. 2. Effects on reproduction. J. Dairy Sci. 87 (Suppl. 1):344.

Ordway, R. S., S. E. Boucher, N. L. Whitehouse, C. G. Schwab, and B. K. Sloan. 2009. Effects of providing two forms of supplemental methionine to periparturient Holstein dairy cows on feed intake and lactational performance. J. Dairy Sci. 92:5154-5166.

Osorio, J. S., P. Ji, J. K. Drackley, D. Luchini, and J. Loor. 2013. Supplemental Smartamine M or MetaSmart during the transition period benefits postpartal cow performance and blood neutrophil function. J. Dairy Sci. 96:6248-6263.

Overton, T. R., D. W. LaCount, T. M. Cicela, and J. H. Clark. 1996. Evaluation of a ruminally protected methionine product for lactating dairy cows. J. Dairy Sci. 79:631-638.

Penagaricano, F., A. H. Souza, P. D. Carvalho, A. M. Driver, R. Gambra, J. Kropp, K. S. Hackbart, D. Luchini, R. D. Shaver, M. C. Wiltbank, and H. Khatib. PLoS ONE 8(8): e72302. doi:10.1371/journal.pone.0072302

Phillips, G. J., T. L. Citron, J. S. Sage, K. A. Cummins, M. J. Cecava, and J. P. McNamara. 2003. Adaptations in body muscle and fat in transition dairy cattle fed differing amounts of protein and methionine hydroxy analog. J. Dairy Sci. 86:3634-3647.

Piepenbrink, M. S., and T. R. Overton. 2003. Liver metabolism and production of cows fed increasing amounts of rumen-protected choline during the periparturient period. J. Dairy Sci. 86:1722-1733.

Piepenbrink, M. S., A. L. Marr, M. R. Waldron, W. R. Butler, T. R. Overton, M. Vazquez-Anon, and M. D. Holt. 2004. Feeding 2-hydroxy-4-(methylthio)-butanoic acid to periparturient dairy cows improves milk production but not hepatic metabolism. J. Dairy Sci. 87:1071-1084.

Preynat, A., H. Lapierre, M. C. Thivierge, M. F. Palin, J. J. Matte, A Desrochers, and C. L. Girard. 2009. Influence of methionine supply on the response of lactational performance of dairy cows to supplementary folic acid and vitamin B_{12}. J. Dairy Sci. 92:1685-1695.

Preynat, A., H. Lapierre, M. C. Thivierge, M. F. Palin, N. Cardinault, J. J. Matte, A Desrochers, and C. L. Girard. 2010. Effects of supplementary folic acid and vitamin B_{12} on hepatic metabolism of dairy cows according to methionine supply. J. Dairy Sci. 93:2130-2142.

Sharma, B. K. and R. A. Erdman. 1988a. Effects of high amounts of dietary choline supplementation on duodenal choline flow and production responses of dairy cows. J. Dairy Sci. 71:2670-2676.

Sharma, B. K. and R. A. Erdman. 1988b. Abomasal infusion of choline and methionine with or without 2-amino-2-methyl-1-propanol for lactating dairy cows. J. Dairy Sci. 71:2406-2411.

Shahsavari, A. 2012. The metabolic and reproductive responses of lactating dairy cows to supplementation with choline. Ph.D. Thesis, University of Queensland.

Socha, M. 1994. Determining the methionine requirements of lactating dairy cows. Ph.D. Thesis, University of New Hampshire.

Soltan, M. A., A. M. Mujalli, M. A. Mandour, and M. El-Shinway Abeer. 2012. Effect of dietary rumen protected methionine and/or choline supplementation on rumen fermentation characteristics and productive performance of early lactating cows. Pakistan J. Nutr. 11:221-230.

Sun F., Y. Cao, C Cai, S. Li, C. Yu, and J. Yao. 2016. Regulation of nutritional metabolism in transition dairy cows: energy homeostasis and health in response to post-ruminal choline and methionine. PLoS ONE 11(8): e0160659. doi:10.1371/journal.pone.0160659

Toledo, M., G. M. Baez, E. Trevisol, N. E. Lobos, A. Garcia-Guerra, J. N. Guen, D. Luchini, R. D. Shaver, M. C. Wiltbank. 2015. Effect of top-dressing rumen-protected methionine in lactating Holstein cows II: Fertility and embryo development. J. Dairy Sci. 98 (Suppl. 2):301.

Zahra, L. C., T. F. Duffield, K. E. Leslie, T. R. Overton, D. Putnam, and S. J. LeBlanc. 2006. Effects of rumen-protected choline and monensin on milk production and metabolism of periparturient cows. J. Dairy Sci. 89:4808-4818.

Zenobi, M.G., R. Gardinal, A. L. G. Dias, J. E. Zuniga, R. Moreira, B. A. Barton, J. E. P. Santos, and C. R. Staples. 2016. Effects of rumen-protected choline (RPC) supplementation to periparturient dairy cows did not depend on prepartum energy intake. J. Dairy Sci. (E-Suppl. 1):657.

Zhou, Z., M. Valilati-Riboni, E. Trevisi, J. K. Drackley, D. N. Luchini, and J. J. Loor. 2016. Better postpartal performance in dairy cows supplemented with rumen-protected methionine compared with choline during the peripartal period. J. Dairy Sci. 99:8716-8732.

Zom, R. L., G, J. van Baal, R. M. A. Goselink, J. A. Bakker, M. J. de Veth, and A. M. van Vuuren. 2011. Effect of rumen-protected choline on performance, blood metabolites, and hepatic triacylglycerols of periparturient dairy cattle. J. Dairy Sci. 94:4016-4027.

Figure 1. Pathways for phosphatidylcholine synthesis.

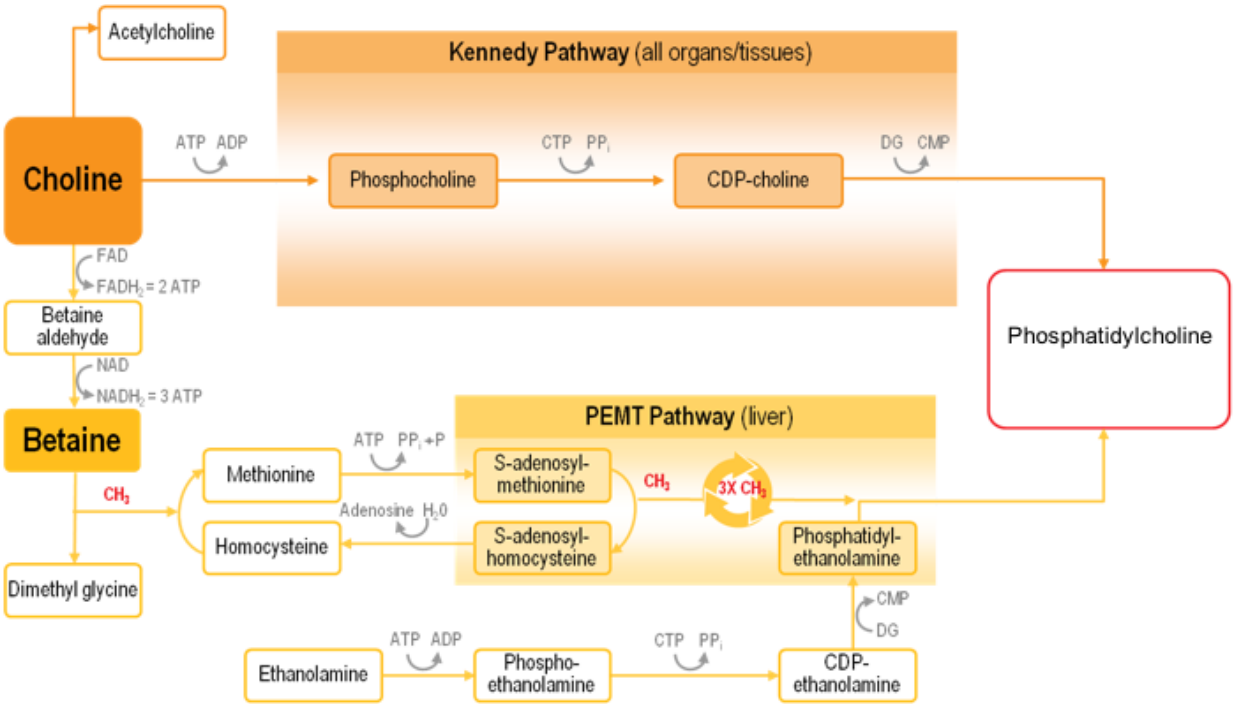


Table 1. Effects of feeding rumen-protected methionine or methionine analog during the transition period on milk yield, milk protein percentage, and protein yield.

Study	Source	Amount and duration	Milk yield, lb/d	Milk protein, %	Milk protein yield, kg/d
Overton et al., 1996	Mepron	0 vs 20 g of Met/d; -10 d to 18 wk	NS	NS	NS
Preynat et al, 2009	Mepron	Met 1.83 vs 2.23% of MP; -3 to +16 wk	NS	2.94 vs 3.04	1.106 vs 1.143
Ordway et al., 2009	Smartamine or Metasmart	SM (0.06\0.10) or MS (0.35\0.54) % of DM Pre/Post; -21 to +140 d	NS	2.72 vs 2.81 (MS) or 2.87 (SM)	NS
Ghorbani et al., 2007	Smartamine	12 (-2 to +2 wk) or 17 g of Met; +3 to +14 wk	NS	2.76 vs 2.93	NR
Osorio et al., 2013	Smartamine /Metasmart	3.4 vs 2.8:1 Lys:Met; -21d to +30d	78.6 vs 86.0 (pooled SM/MS)	3.04 vs 3.22	1.110 vs 1.235
Zhou et al., 2016	Smartamine	3.5 vs 2.9 Lys:Met; -21 d to +30 d	89.0 vs 97.4	3.14 vs 3.32	1.25 vs 1.43
Phillips et al., 2003	HMB	0 vs 20 (pre) or 50 (post) g/d; -21 to +120 d	NS	NS	NR
Piepenbrink et al., 2004	HMB	0 vs 0.09 or 0.18 (pre) or 0.13 or 0.20 (post) % of DM; -21 to +84 d	Inc. Quad 92.4, 99.0, 92.2	NS	NS

Met = methionine; Lys = lysine; MP = metabolizable protein; SM = Smartamine; MS = Metasmart; HMB = methionine hydroxy analog; NS = nonsignificant; NR = not reported; DM = dry matter; d = day; wk = week;

Dietary Choline: A Story Beyond Fatty Liver

C.R. Staples, M. Zenobi, C. Nelson, and J.E.P. Santos.
Department of Animal Sciences. University of Florida.

Introduction

Choline.

Choline has been identified as a required nutrient for many species including humans, chicks, and pigs. Choline is found in low concentrations in most feeds, ranging from 0.04% in corn silage and alfalfa hay to 0.3% in protein sources such as soybean meal and cottonseed meal (DM basis). Its low concentrations in feeds are indicative of the low amounts required by livestock (e.g. 3 g/day for a lactating sow). Although the bovine requirement for choline has yet to be established, the supplementation of choline to dairy cows in transition usually improves milk production and often aids in the reduction of fat in the liver. However, in order for choline to be absorbed in the small intestine of ruminants, the choline must have some protection from degradation by ruminal microbes which degrade dietary choline to methane and acetic acid. Several ruminally-protected choline (**RPC**) products are being marketed commercially to the dairy industry across the world. ReaShure (Balchem Corp., New Hampton, NY) is such a product containing approximately 25% choline chloride. In 16 experiments published since 2003, dairy cows supplemented with an RPC product starting in late gestation (~ 3 weeks prepartum) and continuing into lactation produced an average of 4.4 lb/day more milk or fat-corrected milk compared with cows not supplemented with RPC. Fourteen of the 16 studies reported a numerical increase and 10 reported a statistically significant increase in milk due to RPC supplementation. The need for choline by nonruminants is increased during pregnancy and lactation because of the dam's transport of choline to the fetus during pregnancy and into the milk (Zeisel, 2011). This may well be true for ruminant animals as well.

This increase in cow milk production due to choline supplementation often has been explained through choline's role to improve lipid metabolism by the liver. The liver of the modern dairy cow accumulates fat (triacylglycerol, **TAG**) in the early weeks after calving because of the massive mobilization of adipose tissue for energy use during the extensive period of negative energy balance (**NEB**). The efficiency of the excessively fat liver to manufacture glucose for milk synthesis is compromised resulting in reduced milk yield. Concentrations of choline in the liver decrease dramatically during pregnancy or lactation (Zeisel, 2000). The liver can export some of the fat with the aid of choline. In many studies, simply removing choline from the diet is a way that researchers often create fatty liver in nonruminant species. This reduction in liver TAG caused by feeding RPC to dairy cows helps explain the positive milk responses so commonly reported in the literature. In addition, part of the positive milk response could result from supplemental choline sparing glucose from oxidation for energy so that more is

[1] Contact: 2250 Shealy Drive, Gainesville, FL 32611, Telephone: (352) 392-1958 ext 253, E-Mail: chasstap@ufl.edu

available for synthesis of milk lactose. This may come as a result of methionine contributing to greater carnitine synthesis causing greater oxidation of NEFA rather than glucose for energy.

The many benefits of adequate dietary choline have been identified to a much greater extent in nonruminants compared with ruminants. These include choline's role in 1) the proper development in utero of fetal progenitor cells improving brain and memory development (Zeisel, 2011), 2) reduced risk of birth defects in human babies born from mothers consuming choline-adequate diets (Zeisel, 2011), 3) reduced subclinical fatty liver or muscle damage in adult people (Zeisel and da Costa, 2009), 4) reduced susceptibility to infection of rat pups born from choline-adequate dams (Gebhardt and Newberne, 1974), improved growth rate of rat pups nursing choline-supplemented dams (Dallschaft et al., 2015), and improved maternal immune function of rats (Dallschaft et al., 2015), to name a few.

Prepartum energy intake.

Proper body condition at calving is important for optimal milk yield. Thin cows lack the energy reserves to support needed energy for milk synthesis during the inevitable negative energy state whereas fat cows are often poor eaters and experience greater states of negative energy postpartum resulting in even greater fatty liver, reduced milk yield, and poor reproductive performance. Excessive fat reserves are oftentimes hidden from view; fat that is stored in the abdomen around the intestines and the kidneys is not considered when one is condition-scoring cows. Excessive energy intake during the dry period can build up this abdominal (visceral) fat without changing the overall body condition of the cow (Drackley et al., 2014). Nonlactating and nonpregnant Holstein cows were fed a lower energy diet (0.61 Mcal/lb) of 41% wheat straw and 28% corn silage or a higher energy diet (0.735 Mcal/lb) of 0% wheat straw and 50% corn silage (DM basis) in ad libitum amounts. After 8 weeks on the 2 diets, body condition was the same, 3.47 vs. 3.52, respectively. Upon slaughter, it was discovered that the cows fed the lower energy diet had 56 fewer pounds of abdominal fat (70 vs. 126 lb). Feeding prepartum diets that better match the energy requirement for maintenance and pregnancy is the "just right" (a.k.a. the "Goldilocks") approach. Eating the porridge at the right temperature, sitting in the chair that is the right height, and sleeping in the bed that is the most comfortable is not extreme to either side. Likewise, underfeeding or overfeeding energy to pregnant cows during the whole dry period ends up damaging cow performance postpartum. In the last 2 decades of research in this area of transition cow feeding, formulating well-balanced diets containing substantial proportions of low quality forages such as wheat straw has often but not always been beneficial to postpartum performance (Drackley, 2016). Too often, cows are overfed during the dry period. This approach may not appear to be harmful because body condition appears "normal" but research indicates that dangers lurk like the fury of a moma bear.

Experimental Hypothesis and Approach

Because overfeeding energy during the dry period often leads to fatty liver and because choline plays a key role in improving the liver's management of fat, it was hypothesized that choline supplementation would most benefit those dairy cows overfed energy during the dry period.

Ninety-six pregnant, nonlactating multiparous Holstein cows (University of Florida) were assigned to 1 of 4 dietary treatments on the day of 'dry off' (~7 weeks prior to expected calving date). Dietary treatments were arranged in a 2 × 2 factorial. One factor was RPC (ReaShure, Balchem Corp., New Hampton, NY) top-dressed once daily at 0 or 60 g/day per cow from 21 days prior to expected calving date through 21 days postpartum. The second factor was diets of 0.74 (excess energy) or 0.64 (maintenance energy) Mcal of NE_L/lb of dietary DM fed in ad libitum amounts for the whole dry period. Therefore, the 4 treatments were maintenance energy intake without RPC (**MNE**) or with RPC (**MNE+C**) and excess energy intake without RPC (**EXE**) or with RPC (**EXE+C**). Chopped wheat straw (< 2 inches), corn silage, and triticale silage were adjusted to formulate to the targeted energy density of the prepartum diets. Wet brew was added to the TMR (16.7% of dietary DM) to minimize sorting by the cows managed in a Calan gate system. At the time of enrollment, parity (1.9), 305-day mature equivalent milk production (26,701 lb), body condition score (3.55), or body weight (1622 lb) did not differ between the 4 groups of cows. After calving all cows were fed the same basal diet (0.76 Mcal NE_L per lb and 16.0% CP, DM basis) through 15 weeks postpartum when the trial ended. Diets were formulated to have methionine at 2.3% of metabolizable protein and a lysine-to-methionine ratio of 2.9 prepartum and 3.1 postpartum. Measurements taken included intake of feed, body weight and condition, yield and IgG content of colostrum, health disorders, milk production and composition, triacylglycerol content of liver via biopsy, uterine health assessments, selected metabolites and immune responses in blood, and pregnancy to timed artificial insemination.

Data were analyzed using the MIXED procedure of SAS version 9.4 (SAS/STAT, SAS Institute Inc., Cary, NC). The REPEATED statement was used for dependent variables measured over time. Models included the fixed effects of energy intake prepartum (excess vs. maintenance), RPC (with vs. without), interaction between energy intake prepartum and RPC, day or week of measurement, and all 2- and 3-way interactions. Cow was nested within treatment and was the error term for testing the effects of treatment. Data were transformed to achieve normality if needed before analyses. Binary data were analyzed by logistic regression using the GLIMMIX procedure of SAS. Time to event such as interval to pregnancy by 210 DIM was analyzed with Cox's proportional hazard regression model using the PHREG procedure of SAS. Statistical significance was considered at $P \leq 0.05$ and tendency was considered at $0.05 < P \leq 0.10$.

Experimental Results and Discussion

For nearly every dependent variable, the influence of each main treatment effect was independent. That is, the effect of choline was the same if the cow was fed the lower energy diet or the greater energy diet prepartum. Likewise, the effect of prepartum energy intake was the same regardless of whether the cow was supplemented with choline. Therefore, the main effects of prepartum energy intake and choline will be presented separately.

Effects of prepartum energy intake.

Prepartum responses. Body condition score from dry-off to calving was unchanged. Mean DM intake during the last 15 days of gestation (mean of 23.7 lb/day) did not differ due to energy density of the diets. However intake of energy did differ between the 2 groups as planned. Two weeks prior to calving, cows fed the EXE diet were consuming energy at 140% of their requirement for maintenance and pregnancy whereas cows fed the MNE diet were eating at 110% of their requirement (NRC, 2001). The pattern of NE_L intake over the last 2 weeks of gestation also differed [$P < 0.01$, energy diet by day interaction (**Figure** 1)]. As reported by many others, intake of energy decreased as parturition approached. However, the intake of NE_L by cows fed the EXE diet decreased at twice the rate compared to that by cows fed the MNE diet, dropping the equivalent to 0.6 vs. 0.3 lb per day or a total of 9 (34%) and 4.5 lb (20%), respectively. As a result of the greater NE_L intake prepartum, mean concentration of nonesterified fatty acids (**NEFA**) tended to be lower (252 vs. 295 µEq/mL, $P < 0.10$) and that of glucose was greater (66.4 vs. 63.5 mg/100 mL, $P < 0.05$) in plasma of cows fed the EXE compared with the MNE diet although values were within the normal range for well-managed prepartum dairy cows.

Postpartum responses. Cows fed the EXE diet prepartum consumed 2.7 lb less feed DM ($P < 0.01$) during the 15-week postpartum period compared with cows fed the MNE diet (50.4 vs. 53.1 lb/day, respectively). This response is rarely significant although numerically lower postpartum DM intake by cows overfed energy prepartum has been reported previously (Holcomb et al., 2001; Dann et al., 2006; Zhang et al., 2015). However, mean production of milk over the first 15 weeks postpartum was not different (91.9 vs. 95.1 lb/day of uncorrected milk yield [$P = 0.25$] and 93.9 vs. 96.2 lb/day of energy-corrected milk yield [$P = 0.38$] for cows consuming EXE and MNE diets, respectively). Concentration of fat (3.88 vs. 3.78%) and true protein (2.95 vs. 2.97%) in milk were not affected by prepartum energy intake. The gross efficiency of converting feed DM into ECM almost reached a significant tendency favoring cows fed the EXE diet (1.90 vs. 1.84 lb of milk per lb of feed intake, $P = 0.11$). This improved gross efficiency of milk from feed came at the cost of body reserves. After body weight of both groups of cows hit a low after 4 weeks of lactation, cows from the EXE treatment simply maintained their body weight the rest of the way whereas cows from the MNE treatment started gaining weight until they put on ~70 lb at 15 weeks postpartum. This greater reliance on body reserves for the milk that was produced by cows fed EXE diets prepartum is reflected in greater mean concentrations of circulating beta-hydroxybutyric acid (**BHBA**; 0.52 vs. 0.43 mmol/L, $P < 0.05$) and NEFA (502 vs. 453 µEq/mL, $P <$

0.10). As a result of greater fat circulating in the blood, the liver of cows fed EXE diets accumulated more TAG fat at 7 (11.1 vs. 8.7% of DM) and 21 (10.1 vs. 7.6% of DM) days in milk compared with cows fed MNE diets prepartum.

Fatty liver is often associated with ketosis and reduced reproductive performance. Incidence of health disorders were recorded although the study lacked sufficient numbers of cows to adequately test the effect of prepartum energy intake. Incidence of diseases/disorders that reached a probability of significance of ≤ 0.20 due to feeding EXE diets included ketosis (16.9 vs. 10.2%) and uterine infection at 40 days in milk (15.2 vs. 7.1%). However excess energy intake prepartum did not influence ovarian cyclicity postpartum either at 26 (45.1 vs. 60.6%) or at 40 (78.7 vs. 82.5%) days in milk compared to cows fed MNE diets as determined by the presence of a corpus luteum detected using ultrasonography. Pregnancy at first AI was 32% for both treatment groups.

Effects of choline supplementation.

Prepartum responses. Although pregnant cows began RPC supplementation at 21 days prior to expected calving date, cows consumed supplemental RPC for only the last 17 days of gestation on average because they calved earlier than expected. Supplementing RPC did not change mean DM intake during the last 15 days (23.1 vs. 24.2 lb DM/day for –RPC and +RPC-fed cows, respectively). Body condition score of cows averaged 3.51 and did not differ due to RPC feeding. Blood concentrations of NEFA and BHBA also were unaffected by RPC supplementation.

Postpartum responses. Yield of colostrum was not affected by RPC supplementation (18.8 vs. 21.8 lb) but colostrum from cows fed RPC had a greater concentration of immunoglobulin G (**IgG**; 78 vs. 57 g of IgG/L). The source of colostrum that was fed to the calves born from the cows on this study was not controlled. Nevertheless, the growth of the calves over the following 12 months of life was affected by being exposed to RPC in utero. Calves born to dams supplemented with RPC tended to be 4.6 lb lighter at birth (84.5 vs. 89.2 lb, $P < 0.10$) but were 31 lb heavier at 12 months of age (739 vs. 7089 lb, $P < 0.05$) thus growing at 0.1 lb/day faster compared to calves born from unsupplemented dams (1.97 vs. 1.87 lb/day). Apart from the colostrum, all calves were managed the same during this time period. Feeding more choline to gestating rats improved the choline status of their pups (Dellschaft et al., 2015). This may hold true for ruminants as well. Choline has been helpful in the diet of nonruminant animals during pregnancy to improve offspring performance (Newberme et al., 1970; Zeisel, 2006). Cai et al. (2014) reported that supplementing sows throughout gestation with betaine (a metabolite of choline; 3 g/kg of diet) may improve hepatic gluconeogenesis in newborn piglets. Specifically, newborn piglets from betaine-supplemented sows had greater serum concentrations of lactic acid and gluconeogenic amino acids including serine, glutamate, methionine and histidine. In addition, liver tissue from these piglets contained greater glycogen concentration (0.16 vs. 0.13 g/g) and PEPCK1 enzyme activity, as well as greater protein expression of several gluconeogenic enzymes, namely, pyruvate carboxylase (PC), cytoplasmic phosphoenolpyruvate carboxykinase (PEPCK1), mitochondrial phosphoenolpyruvate

carboxykinase (PEPCK2), and fructose-1,6-bisphosphatase (FBP1) compared to control piglets. Feeding ruminally-protected choline (RPC) during late gestation to pregnant ruminants may provoke changes in expression of gluconeogenic genes in the liver of pre-ruminants causing long-term positive effects in glucose homeostasis later in ruminant life. Whether this may be true for dairy calves should be investigated in the future.

As occurred in the prepartum period, intake of feed DM postpartum was not affected by RPC supplementation (52.3 vs. 51.1 lb/day) although the 1.2 lb/day numerical increase due to RPC supplementation is the same increase as that reported by Grummer (2012) using a meta-analysis of RPC-feeding studies with lactating dairy cows. Cows supplemented with RPC tended ($P < 0.10$) to produce more milk during the first 15 weeks of lactation (95.9 vs. 91.0 lb/day). This tendency for increased milk yield detected during the first 15 weeks continued for 40 weeks of lactation (81.7 vs. 77.1 lb/day, $P < 0.10$; **Figure** 2). Holstein cows produced nearly 5 more pounds per day of milk for 40 weeks of lactation when supplemented with 15 g of choline chloride for approximately 5.5 weeks over the transition period. This milk increase is similar to that reported by Elek et al. (2008), Janovick et al. (2006), and Lima et al. (2012) and to that reported in the meta-analysis by Grummer (2012). Although concentration of fat (3.82 vs. 3.84%) and true protein (2.95 vs. 2.97%) in milk were not affected by RPC, the yield of both fat and protein tended to be greater by cows fed RPC due to their tendency for greater milk yield. Greater milk yield without a significant increase in feed intake resulted in a greater mean NEB of cows fed RPC over the 15 weeks (-1.18 vs. -0.53 Mcal/day). The pattern of NEB over the 15 weeks postpartum also differed between groups. Cows fed RPC were experiencing a more NEB in weeks 2 (-11.4 vs. -8.9 Mcal/day) and 3 (-8.7 vs. -6.6 Mcal/day) postpartum. No difference in energy balance occurred between groups after cows moved past week 6 (RPC by week interaction, $P < 0.10$). Despite a greater NEB, loss of body weight from calving to week 4 postpartum was not different (101 vs. 83 lb). In addition, mean concentrations of NEFA and BHBA in blood were not affected.

Treatment for ketosis was the only disease/disorder that reached a probability of significance of ≤ 0.20 due to feeding RPC (18 vs. 9% for +RPC vs. –RPC, respectively). Diagnosis of ketosis was based upon ketostix classification of urine BHBA as ‘moderate’ (~40 mg/100 mL) or ‘large’ (80 mg/100 mL). In a field study using more cows (n = 369), primiparous and multiparous cows were fed 15 g/d of RPC from 25 days prepartum to 80 days postpartum (Lima et al., 2012). Yield of fat-corrected milk increased 4 lb/day (98.3 vs. 94.3 lb/day) due to RPC feeding. Cows fed RPC had less morbidity, especially less clinical ketosis (4.7 vs. 13.9% for primiparous cows and 3.5 vs. 9.8% for multiparous cows). Other measures that are indicators of cow health suggest a positive influence of RPC in the current study. Rectal temperature measured at 4, 7, and 12 days in milk decreased linearly from 101.8 to 101.2°F for RPC-supplemented cows whereas that for –RPC cows increased linearly from 101.6 to 101.9°F. A concentration of < 8.5 mg of total Ca/100 mL of blood plasma was used as a definition of subclinical milk fever in blood samples collected at 0, 1, 3, and 7 days in milk (Chapinal et al., 2012; Martinez et al., 2012). Cows fed RPC had greater mean concentrations of Ca

across measurement days (8.72 vs. 8.46 mg/100 mL) and the prevalence of subclinical milk fever (using any of the 4 days of measurement) was reduced ($P < 0.05$) from 52.1 to 31.6%.

The pattern and the mean concentration of TAG in liver over 7, 14, and 21 days in milk was not affected by RPC supplementation (8.2 vs. 7.4% TAG DM basis for +RPC and –RPC, respectively). This lack of effect of RPC on liver TAG is in agreement with Zahra et al. (2006) and Piepenbrink and Overton (2003). However several studies have reported reduced TAG concentrations in the liver of lactating dairy cows in the early postpartum period including Elek et al. (2013), Santos and Lima (2009), and Zom et al. (2011). The TAG values in the current FL study were quite low and may have been less susceptible to TAG reduction by RPC.

The greater NEB of cows fed RPC did not influence the proportion of cows cycling at 26 and 40 days in milk based upon a detectable corpus luteum. However pregnancy at first insemination tended to favor cows fed RPC (41.3 vs. 23.6%, $P < 0.10$) although the proportion of cows pregnant by 40 weeks postpartum did not differ (69.8 vs. 62.5%). In a study conducted at a commercial dairy in California using both primiparous and multiparous cows (Lima et al., 2012), pregnancy rate after the first and second insemination was numerically but not significantly better due to feeding RPC from 25 days pre-calving to 80 days post-calving (59.8 vs. 52.7%).

Summary

Compared with feeding to maintenance, overfeeding energy by 40% during the dry period resulted in a greater decrease in DM intake as day of calving approached. After calving, intake of DM was lower (2.7 lb/day). Yield of milk was 3.2 lb/d less but not statistically different. Concentrations of fat in blood and liver were greater and body weight gain was delayed postpartum. The postpartum performance and metabolic status of multiparous cows was compromised by offering diets formulated to exceed energy needs of the pregnant nonlactating cow during the entire dry period.

Supplementing ruminally protected choline chloride at 15 g/day from approximately 17 days prepartum to 21 days postpartum resulted in greater ($P < 0.10$) yield of milk (4.9 lb/day) and milk components through 40 weeks of lactation, greater NEB at 2 and 3 weeks postpartum without changing TAG in liver, greater concentration and yield of IgG in colostrum, greater pregnancy at first insemination, and better daily gains of body weight by calves from those dams regardless of the amount of energy consumed during the entire dry period. Supplemental protected choline during the transition period may offer additional benefits to the dairy enterprise beyond increased milk production and improved liver health. Improvements in immunity, fertility, and calf growth as detected in this study are intriguing and deserve further attention. If these results are confirmed in future studies, the case for choline as an essential nutrient for high-producing ruminants will be solidified.

References

Cai, D., Y. Jia, H. Song, S. Sui, J. Lu, Z. Jiang, and R. Zhao. 2014. Betaine supplementation in maternal diet modulates the epigenetic regulation of hepatic gluconeogenic genes in neonatal piglets. PloS One, 9(8), p.e105504.

Chapinal, N., M. E. Carson, S. J. LeBlanc, K. E. Leslie, S. Godden, M. Capel, J. E. P. Santos, M. W. Overton, and T. F. Duffield. 2012. The association of serum metabolites in the transition period with milk production and early-lactation reproductive performance. J. Dairy Sci. 95:1301-1309.

Dann, H. M., N. B. Litherland, J. P. Underwood, M. Bionaz, A. D'Angelo, J. W. McFadden, and J. K. Drackley. 2006. Diets during far-off and close-up dry periods affect periparturient metabolism and lactation in multiparous cows. J. Dairy Sci. 89:3563-3577.

Dellschaft, N. S., M. R. Ruth, S. Goruk, E. D. Lewis, C. Richard, R. L. Jacobs, J. M. Curtis, and C. J. Field. 2015. Choline is required in the diet of lactating dams to maintain maternal immune function. Brit. J. Nutr. 113:1723-1731.

Drackley, J. K. 2016. "The "Goldilocks diet" 10 years on…is it still "just right"? in Proc. SW Nutrition Conf. Tempe, AZ. pp. 129-141.

Drackley, J. K., R. L. Wallace, D. Graugnard, J. Vasquez, B. F. Richards, and J. J. Loor. 2014. Visceral adipose tissue mass in nonlactating dairy cows fed diets differing in energy density. J. Dairy Sci. 97:3420-3430.

Elek, P., T. Gaal, and F. Husveth. 2013. Influence of rumen-protected choline on liver composition and blood variables indicating energy balance in periparturient dairy cows. Acta. Veterinaria Hungarica 61:59-70.

Elek, P., J. R. Newbold, T. Gaal, L. Wagner, and F. Husveth. 2008. Effects of rumen-protected choline supplementation on milk production and choline supply of periparturient dairy cows. Animal 2:1595-1601.

Gebhardt, B. M., and P. M. Newberne, 1974. Nutrition and immunological responsiveness: T-cell function in the offspring of lipotrope- and protein-deficient rats. Immunology 26:489.

Grummer, R. R., 2012. Choline: A limiting nutrient for transition dairy cows. Pages 22-27 *in* Proc. Cornell Nutr. Conf. Cornell University, Syracuse, NY.

Holcomb, C. S., H. H. Van Horn, H. H. Head, M. B. Hall, and C. J. Wilcox. 2001. Effects of prepartum dry matter intake and forage percentage on postpartum performance of lactating dairy cows. J. Dairy Sci. 84:2051-2058.

Janovick Guretzky, N. A., D. B. Carlson, J. E. Garrett, and J. K. Drackley. 2006. Lipid metabolite profiles and milk production for Holstein and Jersey cows fed rumen-protected choline during the periparturient period. J. Dairy Sci. 89:188-200.

Lima, F. S., M. F. Sa Filho, L. F. Greco, and J. E. P. Santos. 2012. Effects of feeding rumen-protected choline on incidence of diseases and reproduction in dairy cows. Vet. J. 193:140-146.

Martinez, N., F. S. Lima, R. S. Bisinotto, L. F. Greco, E. S. Ribeiro, F. Maunsell, K. N. Galvão, C. A. Risco, and J. E. P. Santos. 2012. Evaluation of peripartal calcium status, energetic profile, and neutrophil function in dairy cows at low or high risk of developing uterine disease. J. Dairy Sci. 95:7158-7172.

Newberne, P. M., R. B. Wilson, and G. Williams. 1970. Effects of severe and marginal maternal lipotrope deficiency on response of postnatal rats to infection. Brit. J. Exp. Path. 51:229.

Piepenbrink, M. S., and T. R. Overton. 2003. Liver metabolism and production of cows fed increasing amounts of rumen-protected choline during the periparturient period. J. Dairy Sci. 86:1722-1733.

NRC, 2001. Nutrient requirements of dairy cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

Santos, J. E. P., and F. S. Lima. 2009. Feeding rumen-protected choline to transition cows. Pages 149-159 in Proc. Florida Ruminant Nutrition Symposium. Univ. FL, Gainesville.

Zang, Q, H. Su, F. Wang, Z. Cao, and S. Li. 2015. Effects of energy density in close-up diets and postpartum supplementation of extruded full-fat soybean oil on lactation performance and metabolic and hormonal status of dairy cows. J. Dairy Sci. 98:7115-7130.

Zeisel, S. H. 2000. Choline: An essential nutrient for humans. Nutrition 16:669-671.

Zeisel, S. H. 2006. Choline: Critical role during fetal development and dietary requirements in adults. Annual Rev. Nutr. 26:229 -250. http://doi.org/10.1146/annurev.nutr.26.061505.111156

Zeisel, S. H. 2011. The supply of choline is important for fetal progenitor cells. Cell & Developmental Biol. 22:624-628.

Zeisel, S. H., and K. da Costa. 2009. Choline: An essential nutrient for public health. Nutr. Rev. 67:615-623.

Figure 1. Effect of Prepartum Diet on Energy Intake

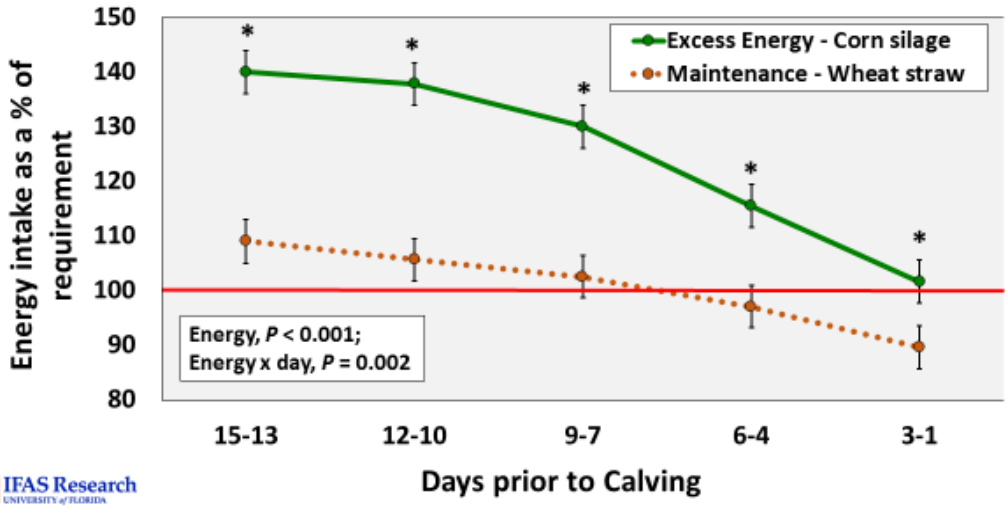
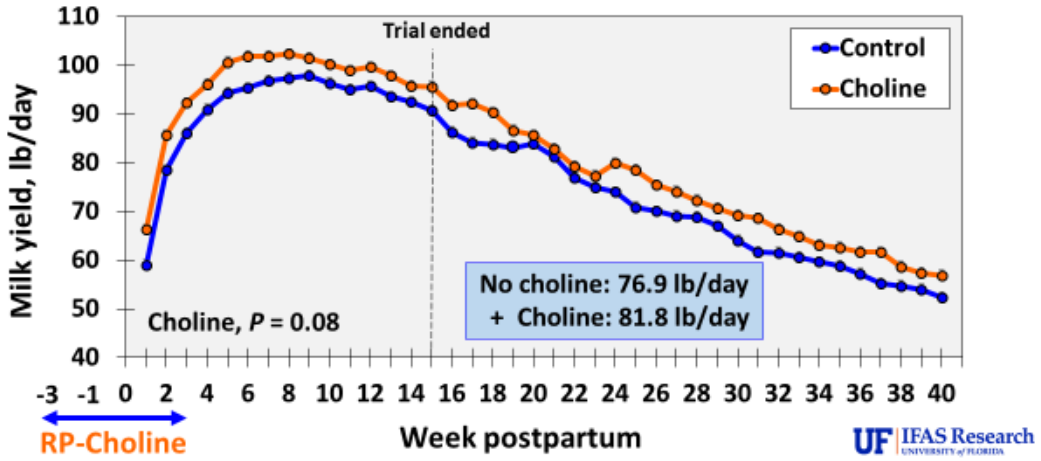


Figure 2. Positive Benefits of Ruminally-Protected Choline Continued After Supplementation Ceased – 40 Weeks



Cows Need Both C16 and C18 Fatty Acids

J. R. Loften[1], M. D. Sellers, and J. G. Linn
Milk Specialties Global, Eden Prairie, MN

Introduction

During the past decade, fatty acid (**FA**) research has been focused on discovering the optimal combination of FA to feed to lactating cows for the purpose of improving milk yield (**MY**) and milk components. Most long chain fatty acid (**LCFA**) supplements contain either combinations of palmitic (**C16:0**) and oleic acids (**C18:1**), highly enriched C16:0 (>80%), or C16:0, stearic acid (**C18:0**), and C18:1. These supplement categories have substantial published research trials over the past 30 years. Nutritionists, researchers, and dairymen are continuing to search for the most optimal combination of these three LCFA. New information continues to enhance our knowledge of the metabolism and utilization of these LCFA for the purpose of improving MY, milk fat (**MF**), milk protein (**MP**), and reproduction by improving energy balance (**EB**) in early lactation. These LCFA are intimately involved in the metabolism of the lactating cow and have specific functions in the production of milk and milk components. Palmitic acid has been shown to improve milk fat % and yield. However, supplementing C16:0 has no effect on MY, body weight gain or body condition score (**BCS**). Stearic acid has been observed to have a positive influence on dry matter intake (**DMI**) and the yield of milk, MF, MP, and milk lactose. Combinations of C16:0 and C18:0 have been shown to improve MY, milk components, and improve EB in early lactation. Thus, the importance in discovering the proper ratio and feeding rates of these LCFA to improve performance is of interest.

Fatty Acids That Enter The Rumen Are Not What Leaves The Rumen

Palmitic and C18:0 are saturated LCFA which have little effect on ruminal microbial populations and are considered rumen inert. Wu et al. (1991) observed quantities of C18:0 leaving the rumen were several fold higher than the amount fed, while C16:0 is similar to the amount fed. Loor et al. (2004) observed that while C18:0 was only 2.1 to 2.4% of the total FA fed in a high (65%) or low concentrate (35%) diet, the amount flowing into the duodenum was ~25 times higher than the amount fed. Stearic acid accounted for 46 to 39% of the total FA flow leaving the rumen in the low- and high-concentrate diets, respectively. The flow of C18:0 from rumen to duodenum is an evolutionary phenomenon that emphasizes the importance of C18:0 to the lactating cow. Substantial microbial biohydrogenation of mono and polyunsaturated C18 fatty acids (**PUFA**) leads to the several fold increase in duodenal C18:0. While much emphasis has been placed on reducing biohydrogenation of PUFA to positively affect milk FA composition, little research has been conducted to determine just how important C18:0 is to the metabolism of the lactating cow as well as the dual presence of C16:0

[1] Contact information: jloften@milkspecialties.com

and C18:0 in the diet.

Digestibility of C16:0 and C18:0

When entering the abomasum, most FA are calcium, potassium, and sodium salts and mixed in an insoluble particulate phase of feed particles and microbial cells. These salts are dissociated and protonated to a great extent in the abomasum due to low pH, and enter the duodenum mostly as non-ionized free FA (**FFA**). These FFA, if not absorbed, may reform as salts as pH increases in the duodenum and ileum. Several research trials and reviews have reported the digestibility values for C16 and C18 LCFA. These are summarized in **Table 1**. The apparent digestibility of C16:0 and C18:0 is very similar and averages 79.6% and 78.3% respectively. Bauman et al. (2003) concluded that the rumen outflow of lipids are predominantly FFA and differences in the digestibility of individual fatty acids in the small intestine are negligible. Thus, the composition of FA absorbed in the small intestine is similar to the composition of FA leaving the rumen. Boerman et al. (2015) reported in their meta-analysis that C16:0 and C18:0 have similar digestibility averaged across published studies. However, as the quantity of C18:0 increased in the duodenum, the corresponding digestibility declined linearly. Loften et al. (2014) summarized that even if the percentage absorption of C18:0 is decreased at high flows into the duodenum, it likely has limited significance because more C18:0 is present in the small intestine than any other FA and, therefore, the quantity absorbed relative to other FA is always much greater.

Metabolism in Tissues

Ruminant adipose tissue is active in both lipogenesis and lipolysis. The major FA concentrations in adult ruminant adipose tissue are C18:1, followed by C16:0, and then C18:0. Choi et al. (2013) reported the C16:0, C18:0, and C18:1 adipose tissue concentrations as 27.9%, 10.4%, and 42.9%, respectively in feedlot steers fed a low fat basal diet. Douglas et al. (2007) reported the C16:0, C18:0, and C18:1 adipose tissue concentrations as 27.0%, 10.7%, and 48.6% in dairy cows prior to calving. The FA in adipose cells come from both diet and de novo synthesis.

Determining the amount of dietary FA uptake in adipose tissue is difficult due to the flux between lipogenesis and lipolysis occurring constantly in a dynamic state. Summers et al. (2000) estimated that slightly more than 10% of saturated FA were stored in human adipose tissue compared to those that were consumed. Mitchaothai et al. (2007) fed finishing swine a diet containing 5% sunflower oil for 13 weeks resulting in the consumption of 1.24 kg and 0.21 kg of C16:0 and C18:0, respectively. They observed 3.75 kg and 2.39 kg of C16:0 and C18:0 deposited in adipose tissue. The ratio of FA deposition:FA intake for C16:0 was 3:1, whereas C18:0 was 11.9:1. These results in monogastrics indicate that dietary FA may be found in adipose tissue, but the majority of FA deposited in adipose tissue is from de novo synthesis. This is true in ruminants as well. The basic building block for de novo lipogenesis is acetyl-CoA which is derived primarily from acetate and glucose (Hellerstein et al.,1996; Vernon, 1981).

Choi et al. (2013) fed either 1) no added lipid, 2) 3% palm oil, 3) or 3% soybean oil to finishing steers to determine if fat sources differing in FA composition would alter FA composition of adipose tissue. The results are illustrated in **Table 2**. Steers fed 3% palm oil or 3% soybean oil did not show an increase in C16:0 concentration in subcutaneous adipose tissue. The only significant change in FA composition was C18:0. However, C18:0 increase in adipose tissue is likely due to C16:0 elongation to C18:0, and because stearoyl-CoA desaturase activity, which converts C18:0 to C18:1, was inhibited by C16:0 (Choi et al., 2013). Synthesis of FA beyond C16:0 does not occur in ruminant adipose tissue, but through a family of elongation enzymes (**ELOV**), C18:0 is produced from C16:0. Stearic acid is then desaturated to C18:1 by the enzyme stearoyl-CoA desaturase. The primary purpose of desaturation is to regulate fluidity of adipose cells from a buildup of high melting point (solid) C18:0 and loss of membrane integrity. Thus, C18:1 is the predominant FA stored in ruminant adipose tissue. Burns et al. (2012) found C16:0 and C16:1 to be regulators of lipogenesis, desaturation, and apoptosis in adipose cells. Thus, C16:0, C18:0, and C18:1 provide the structure of the tissue while maintaining the fluidity of adipocytes preventing their premature apoptosis. The practical implication of decreased adipogenesis with higher amounts of C16:0 through either dietary sources and/or de novo synthesis in adipose cells is potential weight and body condition loss. In short term feeding studies, both Warntjes et al. (2008) and Piantoni et al. (2013) reported numerical decreases in BCS of cows fed C16:0 compared to cows fed control diets.

In the liver, shortly before and after parturition, plasma NEFA concentrations lead to increased hepatic uptake of FA, their subsequent esterification, and accumulation of triglycerides (Grummer, 1993). Douglas et al. (2007) measured the effects of prepartum nutrition on LCFA composition of total lipids in plasma, adipose tissue, and liver, and whether dry period effects persisted (**Table 3**). Hepatic triglycerides (**TG**) contents of C16:0, C18:0, and *cis* C18:1 were similar in the dry period; but, following parturition, C16:0 and *cis* C18:1 increased compared with 45 d prepartum by 58% and 11%, respectively, while C18:0 decreased 42%. Other studies, Rukkwamsuk et al., (2000) and Litherland et al. (2012) found similar results. Mashek and Grummer (2003a) observed no net uptake of C18:0 in the caprine liver when 0.3 m*M* concentrations of C16:0 and C18:0 were perfused into the caudate lobe. They observed that C16:0 uptake was significantly increased compared with C18:0. Mashek and Grummer (2003b) observed C16:0 oxidation doubled when C18:0 was added to bovine cell hepatic cultures compared with C16:0 alone. This may indicate a role for C18:0 in aiding hepatic tissue clear excess C16:0 that collects in hepatic tissue before and after parturition. Loften et al. (2014) concluded that these data indicate that C18:0 does not accumulate in tissues of cows in negative EB and cows preferentially metabolize C18:0 for energy (e.g., βoxidation) in the liver and muscle or secrete large proportions of C18:0 through milk as both C18:0 and C18:1. From these data, Linn and Loften (2015) concluded C18:0 may be better oxidized by the liver or used as an energy source during late prepartum and early postpartum periods than C16:0.

White et al. (2011) suggested that the circulating FA that are characteristically increased in transition cows may contribute to increased expression of pyruvate

carboxylase mRNA to stimulate gluconeogenesis and maintain oxaloacetate for the tricarboxylic acid cycle. Stearic acid was shown to regulate pyruvate carboxylase promoters (P1, P2, and P3) in different tissues, with C18:0 suppressing promoter P1 and enhancing promoter P3 activity simultaneously. These data suggest that C18:0 contributes to the partitioning of energy during periods of upregulated gluconeogenesis, increased hepatic FA supply, or both. This would suggest that C18:0 may spare glucose in early lactation when negative EB occurs.

Mammary Tissue

Palmitic acid and C18:0 are intimately involved in the synthesis of milk and milk fat. Both FA can be oxidized to supply energy for overall synthesis of milk and milk components. Numerous studies in the literature have evaluated fat supplementation to lactating dairy cows; however, most of these studies were with supplements containing mixtures of FA. Very few studies have looked at feeding only a single purified form of a FA. The classic studies of Steele and Moore (1968a,b), Noble et al. (1969), and Steele (1969) were some of the first to look at effects of feeding a purified source of C16:0 on milk yield and milk components. Steele and Moore (1968a) fed 578 g/d of highly purified C16:0, which increased milk fat percentage by 0.86% units and increased the amount of C16:0 in milk fat almost 2-fold, but had no effect on milk yield of 12.2 kg/d for control and 11.8 kg/d for C16:0-supplemented cows. In a later study (Steele, 1969), when 448 g/d of C16:0 was fed as a replacement for starch in diets of lactating cows, milk yield increased by 1 kg/d for cows fed 16:0 compared with control cows. In all 3 studies, feeding C16:0 increased milk fat percentage and yield of C16:0 in milk fat, but concentration and yield of C4 to C14 FA, along with C18:0 and C18:1 in milk fat, decreased. **Table 4** illustrates the effects of C16:0 and C18:0 on milk FA yield. The observed effects of feeding C16:0 at high levels are suppressed de novo synthesis and reduced C18 in milk fat. Noble et al. (1969) concluded that acetyl CoA carboxylase is inhibited by the mammary uptake of LCFA, in this case, primarily C16:0. The more recent studies show similar responses in reducing de novo synthesis of milk FA. The basic effect of feeding highly enriched C16:0 is the 2-4 fold increase in C16:0 in milk fat at the expense of de novo synthesis and C18:0 and C18:1 in milk fat.

Several recent studies have shown improvements in MF% and yield when C16:0 was fed to lactating cows across different production levels. **Table 5** includes an average of 8 research trials with similar design, period length, and diets. The average response measured in these studies reveals that highly enriched palmitic acid improved fat test from 3.70% to 3.87%, reduced DMI by 1.5 lb/d, did not increase MY(0.04 lb./d), reduced MP% from 3.20% to 3.16%, and reduced milk lactose from 4.75% to 4.71%. These data show the ability of feeding highly enriched C16:0 to improve MF% and MF yield. However, the reduction of DMI aids in the explanation of the absence of a MY increase and a reduction of milk lactose % and MP%. In these studies, the absence of improved MY when 428 g/d of C16:0 were fed causes the economic return to be based solely on MF yield.

There have been two recent trials (Boerman and Lock, 2014; Piantoni et al., 2015) where highly enriched C18:0 was fed to lactating cows. Both studies observed significantly higher DMI when C18:0 was fed from 200-700 g/d. The results of the Piantoni et al. (2015) study are shown in **Table 6**. Feeding 500 g/d of a 98% highly enriched C18:0 resulted in significant increases in DMI and yields of milk, MF, MP, lactose, 3.5% FCM, and ECM while not affecting milk component concentration. The intake of enriched C18:0 resulted in a significant increase in de novo, mixed, and preformed FA yields, quite in contrast to previously mentioned C16:0 trials. The authors also observed a significant interaction between production level and C18:0 supplementation. Lactating cows yielding < 60 lb/d of milk showed very little increase in 3.5% FCM, while those producing > 120 lb/d were observed to increase in excess of 10 lb/d. This illustrates the potential glucose sparing effects of C18:0 as indicated by White et al. (2011). Lactating cows requiring higher energy intake and circulating glucose responded with the highest increases in 3.5% FCM when fed enriched C18:0, while low producing cows partitioned energy from milk and MF production to other body functions.

Conclusions

The importance of C16:0 and C18:0 in the production of milk and milk components has been discussed. Each FA has separate functions, metabolism, and utilization. Feeding either FA separately in an enriched form results in different improvements in performance. Results of these studies illustrate the need for both C16:0 and C18:0 in the LCFA supplement to elicit maximum response to lactating cows. Research is underway to determine the optimal ratio of C16:0 to C18:0 in early, mid, and late lactation.

References

Boerman, J. P., S. B. Potts, M. J. VandeHaar, and A. L. Lock. 2015. Effects of partly replacing dietary starch with fiber and fat on milk production and energy partitioning. 2015. J. Dairy Sci. 98 7264 7276.

Boerman, J. P., J. L. Firkins, N. R. St-Pierre, and A. L. Lock. 2015. Intestinal digestibility of long-chain fatty acids in lactating dairy cows: A meta-analysis and meta-regression. J. Dairy Sci. 98:8889–8903.

Boerman J. P. and A. L. Lock. 2014. Milk yield and milk fat responses to increasing levels of stearic acid supplementation of dairy cows. J. Dairy Sci. Suppl. 1. Abstract 1718.

Burns, T. A., S. K. Duckett, S. L. Pratt, and T. C. Jenkins. 2012. Supplemental palmitoleic (C16:1 *cis*-9) acid reduces lipogenesis and desaturation in bovine adipocyte cultures. J. Anim. Sci. 90:3433-3441.

Choi, S. H., G. O. Gang, J. E. Sawyer, B. J. Johnson, K. H. Kim, C. W. Choi, and S. B. Smith. 2013. Fatty acid biosynthesis and lipogenic enzyme activities in subcutaneous adipose tissue of feedlot steers fed supplementary palm oil or soybean oil. J. Anim. Sci. 91:2091-2098.

de Souza, J., J. L. Garver, C. L. Preseault, and A. L. Lock. 2017. Short communication: Effects of prill size of a palmitic acid–enriched fat supplement on the yield of milk

and milk components, and nutrient digestibility of dairy cows. J. Dairy Sci. Article in press.
Doreau, M., and Y. Chilliard. 1997. Digestion and metabolism of dietary fat in farm animals. Br. J. Nutr. 78(Suppl. 1):S15–S35.
Douglas, G. N., J. Rehage, A. D. Beaulieu, A. O. Bahaa, and J. K. Drackley. 2007. Prepartum nutrition alters fatty acid composition in plasma, adipose tissue, and liver lipids of periparturient dairy cows. J. Dairy Sci. 90:2941–2959.
Enjalbert, F., M. C. Nicot, C. Bayourthe, M. Vernay, and R. Moncoulon. 1997. Effects of dietary calcium soaps of unsaturated fatty acids on digestion, milk composition and physical properties of butter. J. Dairy Res. 64:181–195.
Glasser, F, P. Schmidely, D. Sauvant, and M. Doreau. 2008b. Digestion of fatty acids in ruminants: A meta-analysis of flows and variation factors. 1. C18 fatty acids. Animal 2:691–704.
Grummer, R. R. 1993. Etiology of lipid-related metabolic disorders in periparturient dairy cows. J. Dairy Sci. 76:3882–3896.
Hellerstein, M. K., J. Schwarz, and R. A. Neese. 1996. Regulation of hepatic de novo lipogenesis in humans. Annu. Rev. 16:523-557.
Linn, J. G., and J. R. Loften. 2015. Metabolic Fate of Palmitic and Stearic Acids in Dairy Cows. Proc. Minnesota Nutr. Conference.
Lock, A. L., K. J. Harvatine, J. K. Drackley, and D. E. Bauman. 2006. Concepts in fat and fatty acid digestion in ruminants. Pages 85–100 in Proc. Intermountain Nutr. Conf. Utah State Univ., Logan.
Lock, A. L., C. L. Preseault , J. E. Rico , K. E. DeLand , and M. S. Allen. 2013. Feeding a C16:0-enriched fat supplement increased the yield of milk fat and improved conversion of feed to milk. J. Dairy Sci. 96:6650–6659.
Loften, J. R. , J. G. Linn , J. K. Drackley , T. C. Jenkins , C. G. Soderholm , and A. F. Kertz. 2014. Invited review: Palmitic and stearic acid metabolism in lactating dairy cows. J. Dairy Sci. 97:4661–4674.
Loor, J. J., K. Ueda, A. Ferlay, Y. Chilliard, and M. Doreau. 2004. Biohydrogenation, duodenal flow, and intestinal digestibility of *trans* fatty acids and conjugated linoleic acids in response to dietary forage:concentrate ratio and linseed oil in dairy cows. J. Dairy Sci. 87:2472–2485.
Mashek, D. G., and R. R. Grummer. 2003a. Short communication: Net uptake of nonesterified long chain fatty acids by the perfused caudate lobe of the caprine liver. J. Dairy Sci. 86:1218–1220.
Mashek, D. G., and R. R. Grummer. 2003b. Effects of long chain fatty acids on lipid and glucose metabolism in monolayer cultures of bovine hepatocytes. J. Dairy Sci. 86:2390–2396.
Mitchaothai, J., H. Everts, C. Yuangklang, S. Wittayakun, K. Vasupen, S. Wongsuthavas, P. Srenanul, R. Hovenier, and A. C. Beynen. 2008. Digestion and deposition of individual fatty acids in growing-finishing pigs fed diets containing either beef tallow or sunflower oil. J. Anim. Phys. and An. Nut. 92:502–510.
Noble, R. C., W. Steele, and J. H. Moore. 1969. The effects of dietary palmitic and stearic acids on milk fat composition in the cow. J. Dairy Res. 36:375–381.

Piantoni, P., A. L. Lock and M. S. Allen. 2013. Palmitic acid increased yields of milk and milk fat and nutrient digestibility across production level of lactating cows. J. Dairy Sci. 96:7143-7154.
Piantoni, P., C. M. Ylioja, and M. S. Allen. 2015. Feed intake is related to changes in plasma nonesterified fatty acid concentration and hepatic acetyl CoA content following feeding in lactating dairy cows. J. Dairy Sci. 98:6839–6847**.**
Piantoni, P., A. L. Lock, and M. S. Allen. 2015. Milk production responses to dietary stearic acid vary by production level in dairy cattle. J. Dairy Sci. 98:1–12.
Rico, D. E., Y. Ying, and K. J. Harvatine. 2014. Effect of a high-palmitic acid fat supplement on milk production and apparent total-tract digestibility in high- and low-milk yield dairy cows. J. Dairy Sci. 97:3739–3751.
Scollan, N. D., M. S. Dhaona, N. J. Choi, W. J. Maeng, M. Enser, and J. D. Woods. 2001. Biohydrogenation and digestion of long chain fatty acids in steers fed on different sources of lipid. J. Agric. Sci.136:345-355.
Steele, W., and J. H. Moore. 1968a. The effects of a series of saturated fatty acids in the diet on milk-fat secretion in the cow. J. Dairy Res. 35:361–370.
Steele, W., and J. H. Moore. 1968b. The effects of mono-unsaturated and saturated fatty acids in the diet on milk-fat secretion in the cow. J. Dairy Res. 35:353–360.
Steele, W. 1969. The effects of dietary palmitic and stearic acids on milk production yield and composition in the cow. J. Dairy Res. 36:369–373.
Summers, L. K. M., S. C. Barnes, B. A. Fielding, C. Beysen, V. Ilic, S. M. Humphreys, and K. N Frayn. 2000. Uptake of individual fatty acids into adipose tissue in relation to their presence in the diet. Am. J. Clin. Nutr.71:1470–1477.
Vernon, R. G. 1981. Lipid Metabolism in the Adipose Tissue of Ruminant Animals. Progress in Lipid Research. 19:23-106.
Warntjes, J. L., P. H. Robinson, E. Galo, E. J. DePeters, and D. Howes. 2008. Effects of feeding supplemental palmitic acid (C16:0) on performance and milk fatty acid profile of lactating dairy cows under summer heat. Anim. Feed Sci. Technol. 10:241-258.
White, H. M., S. L. Koser, and S. S. Donkin. 2011. Differential regulation of bovine pyruvate carboxylase promoters by fatty acids and peroxisome proliferator-activated receptor-α agonist. J. Dairy Sci. 94:3428–3436.
Wu, Z., O. A. Ohajuruka, and D. L. Palmquist. 1991. Ruminal synthesis, biohydrogenation, and digestibility of fatty acids by dairy cows. J. Dairy Sci. 74:3025-3034.

Table 1. Apparent digestibility of long chain fatty acids from 6 published studies and reviews.

	Published studies						
	Doreau and Chilliard 1997	Enjalbert et al. 1997	Scollan et al. 2001	Lock et al. 2006	Glasser et al. 2008	Boerman et al. 2015	Average
	Fatty acid digestibility %						
C16:0	79	76	92	75	NA	76	79.6
C18:0	77	79	95	72	74	73	78.3
C18:1	85	78	89	80	79	82	82.2
C18:2	83	66	73	78	72	78	75.0
C18:3	76	63	72	77	70	79	72.8

Table 2. Fatty acid composition of subcutaneous adipose tissue of feedlot steers fed palm oil or soybean oil.[1]

Fatty acid %	Control	3% Palm Oil	3% Soybean Oil	*P* value
C16:0	27.9	27.0	26.7	0.09
C18:0	10.4[a]	12.6[b]	12.6[b]	0.02
C18:1	42.9	42.7	42.9	0.22
C18:2	1.84	1.90	2.04	0.18

[a,b] Means in rows not bearing a common superscript differ, $P < 0.05$.
[1] Adapted from Choi et al. (2013).

Table 3. Fatty acid composition of tissues in pre- and post-partum dairy cows.[1]

Tissue	Day relative to parturition			
g/100 g of FA	-45	1	21	65
Adipose				
C16:0	27.0	27.5		
C18:0	10.7	10.8		
C18:1	49.4	48.1		
Liver TG				
C16:0	26.8	42.3[a]	39.0[a]	26.0[b]
C18:0	25.5	10.6[b]	12.2[b]	24.7[a]
C18:1	23.9	26.6[a]	26.6[a]	17.2[b]
Plasma				
C16:0	17.7	18.2[a]	14.5[b]	12.2[c]
C18:0	16.5	15.6[a]	13.9[b]	13.7[b]
C18:1	18.0	19.6[a]	20.1[a]	14.5[b]

[1] Adapted from Douglas et al. (2007).

Table 4. Effects of feeding C16:0 and C18:0 on milk fatty acid yield.

		Fatty acid fed, g/d	Milk fatty acid yield, g/d		
			De novo	Mixed	Preformed
Steele and Moore 1968a					
	Control	0	82	152	78
	C16:0	578	62	297	86
	C18:0	564	83	133	103
Noble et al. 1969					
	Control	0	143	206	106
	C16:0	448	110	338	112
	C18:0	448	110	138	228
Recent trials[1]					
	Control	0	338	127	436
	C16:0	428	305	543	414

[1] Trials included reported milk fatty acid composition and yield (Piantoni et al., 2013; Lock et al., 2013; Rico et al., 2014; Boerman et al., 2015; and de Souza et al., 2017.)

Table 5. Effect of feeding palmitic acid supplements to lactating cows and milk composition from 8 trials utilizing similar design. **[1]**

		Measures				
Treatment	C16:0 Intake lb/d	DMI lb/d	Milk yield lb/d	Milk fat %	Lactose %	Milk protein %
Control	0	58.3	84.24	3.70	4.75	3.20
Palmitic acid	428	56.8	84.28	3.87	4.71	3.16
Palmitic acid minus Control	428	-1.5	0.04	0.17	-0.04	-0.04

[1] Studies included Lock et al., 2013; Piantoni et al., 2013, Rico et al., 2014; Garver et al., 2015; Boerman et al., 2015; DeSouza et al., 2017.

Table 6. The effects of feeding highly enriched stearic acid on milk yield, milk components, and milk fatty acid yield in lactating dairy cows.[1]

Item		Control	Stearic acid[a]	+/-	*P* value
DMI	lb/d	55.4	57.4		<0.01
Yield					
Milk	lb/d	84.7	88.4	3.7	0.02
Milk fat	g/d	1350	1420	70	<0.01
Milk protein	g/d	1140	1190	50	0.02
Lactose	g/d	1870	1960	90	0.02
3.5% FCM	lb/d	84.0	89.1	5.1	<0.01
ECM	lb/d	84.0	88.2	4.2	<0.01
Composition					
Milk fat	%	3.60	3.59	-0.01	NS
Milk protein	%	3.00	2.99	-0.01	NS
Lactose	%	4.83	4.86	0.03	NS
Milk fatty acids					
De novo	g/d	344	359	15	<0.0001
Mixed	g/d	451	461	10	<0.01
Preformed	g/d	352	393	41	<0.001
TOTAL	g/d	1147	1213	66	<0.01
Transfer efficiency	%		12.9%		
Total FA digestibility	%	76.1	56.6	-19.50	<0.0001
16 C	%	76.2	75.8	-0.40	0.79
18 C	%	79.1	55.3	-23.80	<0.0001

[a] Included in diet at 2% of the DMI or 522 g/d of 98% C18:0 per day.
[1] Adapted from Piantoni et al. (2015).

Effects of Supplementation of a Combination of Palmitic and Stearic Acids on Milk and Component Production: A Meta-Analysis

M. D. Sellers[1], T. L. Harris, and J. R. Loften
Milk Specialties Global Animal Nutrition, Eden Prairie, MN

Introduction

Supplementing the lactating cow ration with a high-energy fat source is a widely adopted strategy that is commonly used to improve energy intake, milk and component production, and reproductive efficiency. A wide array of fat sources have been fed to lactating cattle in recent years including oilseeds such as cottonseed and soybeans, animal fats such as tallow, palm oil products, and various modified fat sources that have been designed to reduce or eliminate availability of unsaturated fatty acids to biohydrogenation in the rumen (Rabiee et al., 2012).

Previous authors have used meta-analytical methods as a means to determine productive and reproductive responses to specific types of supplemental fat or to dietary fat in general. Allen (2000) investigated effects of fat source, fatty acid chain length, degree of fatty acid saturation, and fatty acid esterification on dry matter intake (**DMI**) in lactating cows, and concluded that DMI is affected differently by varying fat sources, and that DMI decreases with increasing proportion of unsaturated fatty acids in the diet. Rabiee et al. (2012) used meta-analysis and meta-regression to determine the effects of supplementation with fats on milk production and components by dairy cows. Five groups of fats were evaluated including tallows, calcium salts of palm fat (Megalac; Church and Dwight Co. Inc., Princeton, NJ), oilseeds, prilled fat, and other calcium salts. The authors concluded that fat supplementation did improve milk yield (**MY**), but the results were heterogeneous across fat groups. All fat groups aside from prilled fats decreased DMI. Several fat groups were also shown to decrease milk fat (**MF**) percentage, while no fat groups influenced milk protein (**MP**) production. Rodney et al. (2015) investigated the relationship between dietary fat and fertility in dairy cattle. The authors concluded that, overall, inclusion of fat in the ration does improve fertility, with varying conclusions for oilseeds, calcium salts of fatty acids, tallow, and conjugated linoleic acid. Most recently, de Souza et al. (2016) conducted a meta-analysis and meta-regression to determine the effects of highly enriched palmitic acid supplements in late lactation dairy cows. The authors reported that MF percentage, MF yield, NDF digestibility, and fatty acid digestibility were increased with palmitic acid feeding; however, MY, DMI, body weight, and body condition score were unaffected by palmitic acid supplementation.

While the above-mentioned studies provide a thorough explanation of some general effects of dietary and supplemental fat on DMI, production, and reproduction, they do not thoroughly explore these topics in regards to supplementation with a

[1]Corresponding author. Email: msellers@milkspecialties.com

combination of palmitic (C16:0) and stearic (C18:0) fatty acids. The paper by Allen (2000) limits inferences on DMI to oilseeds, unprocessed animal fat, hydrogenated triglycerides and fatty acids, and calcium salts of palm fatty acids. The paper by Rabiee et al. (2012) investigates prilled fats but gives no inference into effects of specific fatty acid profiles. Moreover, Rabiee et al. (2012) opted to exclude crossover and Latin square designs from their analysis, so only 3-4 prilled fat comparisons were included in their analysis. The paper by Rodney (2015) eschews prilled fats altogether. Finally, the de Souza et al. (2016) paper investigated the effects of highly enriched palmitic acid products alone, and did not include blended C16:0 and C18:0 supplemental fats in the analysis. With this in mind, the objective of the current analysis was to use meta-analytic methods to examine intake, milk production, milk component, and efficiency responses when lactating cows were supplemented with a prilled fat containing a blend of C16:0 and C18:0 fatty acids.

Materials and Methods

Selection Criteria

The initial selection criteria for inclusion in the primary data set were studies that reported DMI, MY, and milk component concentration and yield measurements in lactating dairy cows when a diet containing no added fat was compared to a diet containing supplemental fat in the form of prilled free fatty acids containing a blend of C16:0 and C18:0 fatty acids. Studies that did not report a measure of variability were then excluded from the data set as recommended by Borenstein et al. (2009). Although Lean et al. (2009) caution against using studies with crossover or Latin square designs due to potential carryover effects and effects of stage of lactation, the authors opted to include these studies, as the number of studies meeting criteria for analysis decreases drastically if these study types are excluded, and the goal of the current analysis was to summarize all available data. The final data set consisted of 25 studies comprising 73 treatment means published in peer-reviewed journals. The means included 39 treatments containing supplemental fat and 34 treatments that did not contain supplemental fat. Descriptive information on the individual studies and treatments included in the data set are reported in **Table 1**.

Data Extraction

Data extracted from qualifying studies included journal, year of publication, authors, trial design, length of trial feeding period, number of cows in control and treatment groups, amount of fat supplemented (g/d and % of dietary DM), DMI (kg/d), net energy (**NE**) intake (Mcal/d), MY (kg/d), MF percentage and yield (kg/d), MP percentage and yield (kg/d), milk lactose (**ML**) percentage and yield (kg/d), 3.5% fat-corrected milk (**FCM**) yield (kg/d), and ratio of 3.5% FCM to DMI (kg/kg per d). A measure of variation (**SD or SE**) was also recorded for each production variable.

Statistical Analysis

All statistical analysis was performed using R statistical software (R Core Team, 2016) and all meta-analysis was performed using the ‘metafor’ package in R

(Viechtbauer, 2010) following guidelines set forth by Lean et al. (2009) and Borenstein et al. (2009). Dry matter intake, NE intake, MY, milk component concentration and yield, 3.5% FCM, and 3.5% FCM to DMI data were analyzed via raw mean difference, which was calculated by subtracting the mean for the control group from the mean for the treatment group. Resulting positive raw mean differences favored treatment groups whereas resulting negative raw mean differences favored control groups. In cases where separate standard deviation or standard errors were reported for control and treatment cows, the appropriate designation for each was recorded for the meta-analysis. If only pooled standard deviations or standard errors were reported, then the pooled version of each was recorded. As all included studies vary in terms of days in milk, diet composition, genetics, etc., the authors opted to use random effects models utilizing the inverse of the variance for weighting as recommended by Borenstein et al. (2009). Estimates of effect size, 95% confidence intervals, and statistical significance of effect size were estimated for each production response. P-values corresponding to effect size significance were estimated using the method of Knapp and Hartung (2003), which provides more conservative estimates when number of studies is small. Mean differences and associated confidence intervals were visualized using forest plots (not shown).

Variation among studies was quantified using the I^2 statistic and assessed for statistical significance using a chi-square test of heterogeneity (Borenstein et al., 2009). The I^2 statistic estimates the proportion of total variation in effect size estimates that is due to heterogeneity. Negative I^2 values were adjusted to 0 so that all I^2 estimates were between 0 and 100 percent. An I^2 value greater than 50 percent may be indicative of substantial heterogeneity (Rabiee et al., 2012).

Publication bias was assessed visually via funnel plots (not shown). Briefly, a funnel plot is a scatter plot of effect size estimates versus their respective estimates of precision. If many large and small studies have been conducted, small, imprecise studies should be scattered around the average effect size, and studies should narrow in on the average effect size as study size and precision increase resulting in a symmetrical ‘funnel’ of data points. If publication bias exists (negative or unfavorable studies tend to not be published), the plot will appear asymmetrical with a large gap at the bottom of the plot.

Results / Discussion

Data Review and Description

All data extracted and analyzed in the meta-analysis are described in **Table 1**. As shown, multiple studies had more than one mean comparison due to multiple fat supplementation levels, changes in other dietary parameters, or similar circumstances that allowed for such. Mean comparisons were performed between control diets and treatment diets within studies that only differed in supplemental fat inclusion. Data were excluded due to non-reported estimates of variance (SE or SD) and/or differing compositions of diets in the control and treatment groups. Tests of heterogeneity, the I^2 statistic and resulting χ^2 *P* – value are reported in **Table 2**. The I^2 statistic was ≥ 44 for

all response variables except 3.5% FCM, indicating that moderate to large variation existed among mean differences for most variables, likely attributable to differences among studies in breed, stage of lactation, diet composition, reproductive status, etc. The χ^2 ($\alpha = 0.10$ due to low power) test indicated that variation among mean differences was greater than 0 for all variables. Visual analysis of funnel plots suggested minimal to no presence of publication bias.

Production Outcomes

The effects of supplementation with a combination of C16:0 and C18:0 fatty acids on DMI and NE intake, milk production, milk composition, milk component yield, and 3.5% FCM feed efficiency are shown in Table 2. The weighted average supplemental fat intake for each variable is indicated and ranged from 524 g to 645 g, well in excess of typical supplemental fat feeding rates observed on most commercial dairies. Supplementation with a combination of C16:0 and C18:0 fatty acids did not reduce DMI (-0.06 kg/d; *P* = 0.7481). Allen (2000) reported linear reductions in DMI with increasing inclusion of oilseeds, unprocessed animal fat, and calcium salts of palm fatty acids, but failed to detect a relationship between inclusion of hydrogenated fats and DMI reduction, and speculated that the observed differences in DMI reduction may be due to differences in fatty acid chain length and degree of saturation. In agreement, Rabiee et al. (2012) reported that fat supplementation, irrespective of fat source, decreased DMI by 0.875 kg/cow per day. When effects on DMI were analyzed individually by fat source, significant reductions in DMI were observed for tallow, Megalac, oilseeds, and other calcium salts but were not observed for prilled fat (-0.088 kg/d; *P* = 0.717). Rodney et al. (2015) reported that DMI was improved with oilseed supplementation (0.15 kg/d), and decreased with supplementation of calcium salts of fatty acids, tallow, and conjugated linoleic acid (**CLA**) (-0.22, -0.72, and -0.63 kg/d for calcium salts, tallow, and CLA, respectively). A significant increase in NE intake was also observed in the current study (2.13 Mcal/d; *P* = 0.0048), and is likely due to increased energy density of the ration with fat supplementation paired with little or no decrease in DMI.

Milk yield and 3.5% FCM yield increased by 1.24 (*P* = 0.0001) and 1.38 (*P* = 0.0004) kg/d, respectively. Reported effects of supplemental fat on MY are variable. Rabiee et al. (2012) reported that milk production improved by 0.244 kg/d (*P* = 0.006) with fat supplementation, but the effect was only significant for Megalac and other calcium salts and was not significant for prilled fat. Contrastingly, Rodney et al. (2015) reported a non-significant increase of 0.33 kg/d with general supplemental fat feeding, and a significant improvement only with feeding of calcium salts of fatty acids (0.73 kg/d). Purified palmitic acid fat supplements were also shown to not improve MY but did improve 3.5% FCM via an increase in milk fat percentage (de Souza et al., 2016).

Milk fat percentage (0.08%; *P* = 0.0093) and yield (0.06 kg/d; *P* = 0.0001) both increased with C16:0 and C18:0 fat supplementation. This increase is likely attributable to increased fatty acid intake and post-ruminal absorption. Moreover, feeding highly saturated fat sources such as a combination C16:0 and C18:0 has little to no negative impact on rumen VFA production or milk fatty acid synthesis in the mammary gland compared with unsaturated fatty acids. Rabiee et al. reported a similar improvement in

MF percentage (0.096%) and MF yield (0.062 kg/d) with prilled fat supplementation, while Rodney et al. (2015) found no change in MF percentage or yield with fat supplementation regardless of source.

Milk protein percentage was not different (-0.02%, $P = 0.3363$) but MP yield was increased (0.03 kg/d; $P = 0.0008$) with supplementation of a combination of C16 and C18 fatty acids. Rabiee et al. (2012) reported that prilled fat supplementation did not affect MP percentage (-0.017%; $P = 0.458$) or MP yield (0.009 kg/d; $P = 0.648$), but MP percentage was decreased by all other fat types and was decreased overall with fat supplementation (-0.077%, $P < 0.001$). Contrastingly, Rodney et al. (2015) reported no change in MP percentage or yield with fat supplementation regardless of source.

Supplementation with a combination of C16:0 and C18:0 tended to decrease ML concentration (-0.04%, $P = 0.0612$), but did not affect ML yield (0.05 kg/d; $P = 0.1553$). Other recent meta-analyses did not include ML percentage or concentration as variables of interest.

The amount of 3.5% fat-corrected milk produced per kilogram of feed intake was also improved (0.06 kg/kg per d; $P = 0.024$), an increase that again can be attributed to improved milk and component yields coupled with no change in DMI.

Conclusions

This meta-analysis is intended to summarize the production responses that have been observed when lactating dairy cows were supplemented with a combination of C16:0 and C18:0 fatty acids. The production responses observed across studies are largely heterogeneous, as indicated by moderate to large I^2 values. This analysis did not control for effects of breed, stage of lactation, diet composition, environment, etc. Nonetheless, when all studies are included in the analysis, C16:0 and C18:0 fatty acid supplementation generally had positive effects on production outcomes despite very high levels of fat supplementation. Dry matter intake and NE intake were both improved, as were MY and 3.5% FCM yield. Milk fat percentage increased by 0.08% while MP and ML percentages did not change. Yields of MF and MP were also increased. Supplementation with a combination of C16:0 and C18:0 fatty acids appears to yield significant improvement in production without harming DMI or NE intake, and may be a promising means to improving dairy cow production and energy balance that warrants further investigation.

References

Allen, M. S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J. Dairy Sci 83:1598-1624.

Bernard, J. K., J. J. Castro, and A. F. Kertz. 2012. Performance and metabolic measures of lactating dairy cows fed diets supplemented with mostly saturated or more unsaturated fatty acds. Prof Anim Sci 28:494-501.

Borenstein, M., L. V. Hedges, J. P. Higgins, and H. R. Rothstein. 2009. Introduction to meta-analysis. John Wiley & Sons, Chichester, U.K.
Chan, S. C., J. T. Huber, K. H. Chen, J. M. Simas, and Z. Wu. 1997a. Effects of ruminally inert fat and evaporative cooling on dairy cows in hot environmental temperatures. J. Dairy Sci 80:1172-1178.
Chan, S. C., J. T. Huber, C. B. Theurer, Z. Wu, K. H. Chen, and J. M. Simas. 1997b. Effects of supplemental fat and protein source on ruminal fermentation and nutrient flow to the duodenum in dairy cows. J. Dairy Sci 80:152-159.
de Souza, J., R. J. Tempelman, M. S. Allen, and A. L. Lock. 2016. Production response, nutrient digestibility, and energy partitioning of post-peak dairy cows when palmitic acid-enriched supplements are included in diets: a meta-analysis and meta-regression. J. Dairy Sci 99(E-Suppl. 1):622.
Greco, L. F., M. Garcia, B. L. Artiaga, E. K. Ganda, R. S. Bisinotto, F. S. Lima, N. Martinez, E. S. Ribeiro, A. L. Lock, W. W. Thatcher, C. R. Staples, and J. E. P. Santos. 2012. Effect of supplemental fatty acids on production responses and hepatic fatty acid composition and gene expression of dairy cows fed diets containing low concentrations of fatty acids. J. Dairy Sci 95(Suppl. 2):103.
Knapp, G. and J. Hartung. 2003. Improved tests for a random effects meta-regression with a single covariate. Stat Med 22:2693-2710.
Lean, I. J., A. R. Rabiee, T. F. Duffield, and I. R. Dohoo. 2009. Invited review: Use of meta-analysis in animal health and reproduction: methods and applications. J. Dairy Sci 92:3545-3565.
Moallem, U., M. Katz, A. Arieli, and H. Lehrer. 2007a. Effects of peripartum propylene glycol or fats differing in fatty acid profiles on feed intake, production, and plasma metabolites in dairy cows. J. Dairy Sci 90:3846-3856.
Moallem, U., M. Katz, H. Lehrer, L. Livshitz, and S. Yakoby. 2007b. Role of peripartum dietary propylene glycol or protected fats on metabolism and early postpartum ovarian follicles. J. Dairy Sci 90:1243-1254.
Piantoni, P., A. L. Lock, and M. S. Allen. 2015a. Saturated fat supplementation interacts with dietary forage neutral detergent fiber content during the immediate postpartum and carryover periods in Holstein cows: Production responses and digestibility of nutrients. J. Dairy Sci 98:3309-3322.
Piantoni, P., A. L. Lock, and M. S. Allen. 2015b. Saturated fat supplementation interacts with dietary forage neutral detergent fiber content during the immediate postpartum period in Holstein cows: Energy balance and metabolism. J. Dairy Sci 98:3323-3334.
R Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-9000051-07-0, URL http://www.R-project.org/.
Rabiee, A. R., K. Breinhild, W. Scott, H. M. Golder, E. Block, and I. J. Lean. 2012. Effect of fat additions to diets of dairy cattle on milk production and components: a meta-analysis and meta-regression. J. Dairy Sci 95:3225-3247.
Relling, A. E. and C. K. Reynolds. 2007. Feeding rumen-inert fats differing in their degree of saturation decreases intake and increases plasma concentrations of gut peptides in lactating dairy cows. J. Dairy Sci 90:1506-1515.

Rodney, R. M., P. Celi, W. Scott, K. Breinhild, and I. J. Lean. 2015. Effects of dietary fat on fertility of dairy cattle: A meta-analysis and meta-regression. J. Dairy Sci 98:5601-5620.

Thering, B. J., D. E. Graugnard, P. Piantoni, and J. J. Loor. 2009. Adipose tissue lipogenic gene networks due to lipid feeding and milk fat depression in lactating cows. J. Dairy Sci 92:4290-4300.

Viechtbauer, W. 2010. Conducting meta-analyses in R with the metafor package. J. Stat Soft 36:1-48.

Wang, J. P., D. P. Bu, J. Q. Wang, X. K. Huo, T. J. Guo, H. Y. Wei, L. Y. Zhou, R. R. Rastani, L. H. Baumgard, and F. D. Li. 2010. Effect of saturated fatty acid supplementation on production and metabolism indices in heat-stressed mid-lactation dairy cows. J. Dairy Sci 93:4121-4127.

Weiss, W. P. and J. M. Pinos-Rodriguez. 2009. Production responses of dairy cows when fed supplemental fat in low- and high-forage diets. J. Dairy Sci 92:6144-6155.

Weiss, W. P., J. M. Pinos-Rodriguez, and D. J. Wyatt. 2011. The value of different fat supplements as sources of digestible energy for lactating dairy cows. J. Dairy Sci 94:931-939.

Table 1. Description of studies and treatments included in the analysis

Study	N cows per trt	Treatment name; treatment category (Supplemental Fat % of DM)
Grummer, 1988	4	1. Control; control (0%)
	4	2. LPF (low prilled fat); supplemental fat (3.8%)
	4	3. HPF (high prilled fat); supplemental fat (5.2%)
Schauff and Clark, 1989	4	1. Control (Experiment 1); control (0%)
	4	2. LPF (low prilled fat; Experiment 1); supplemental fat (3.6%)
	4	3. HPF (high prilled fat; Experiment 1); supplemental fat (4.9%)
	6	4. Control (Experiment 2); control (0%)
	6	5. PF (prilled fat; Experiment 2); supplemental fat (2.4%)
Skaar et al., 1989	10	1. Control; control (0%)
	9	2. Fat; supplemental fat (5%)
	10	3. Niacin; control (0%)
	10	4. Fat + niacin; supplemental fat (5%)
Wu et al., 1993	6	1. Control; control (0%)
	6	2. PF (prilled fat); supplemental fat (2.5%)
Wu et al., 1994	6	1. WCS (whole cottonseed); control (0%)
	6	2. WCSPT (whole cottonseed prilled tallow); supplemental fat (2.2%)
	6	3. WCSPT+ (whole cottonseed prilled tallow plus); supplemental fat (4.4%)
Elliott et al., 1995	16	1. High NSC (Experiment 1); control (0%)
	16	2. High NSC plus fat (Experiment 1); supplemental fat (2.5%)
	16	3. Low NSC (Experiment 1); control (0%)
	16	4. Low NSC plus fat (Experiment 1); supplemental fat (2.5%)
	8	5. High NSC (Experiment 2); control (0%)
	8	6. High NSC plus fat (Experiment 2); supplemental fat (2.5%)
	8	7. Low NSC (Experiment 2); control (0%)
	8	8. Low NSC plus fat (Experiment 2); supplemental fat (2.5%)
Elliott et al., 1996	5	1. Control; control (0%)
	5	2. Prilled FA; supplemental fat (5%)
Grum et al., 1996	8	1. Low concentrate; control (0%)
	8	2. Low concentrate plus fat; supplemental fat (3%)
	8	3. High concentrate; control (0%)
	8	4. High concentrate plus fat; supplemental fat (3%)
Chan et al., 1997a	4	1. Medium Fat plus Low Quality Protein; control (0%)
	4	2. High Fat plus Low Quality Protein; supplemental fat (2.5%)
	4	3. Medium Fat plus High Quality Protein; control (0%)
	4	4. High Fat plus High Quality Protein; supplemental fat (2.5%)

Table 1. Description of studies and treatments included in the analysis (cont.)

Study	N cows per trt	Treatment name; treatment category (Supplemental Fat % of DM)
Chan et al., 1997b	6	1. Medium Fat plus Shade; control (0%)
	6	2. High Fat plus Shade; supplemental fat (3%)
	6	3. Medium Fat plus Evaporative Cooling; control (0%)
	6	4. High Fat plus Evaporative Cooling; supplemental fat (3%)
Simas et al. 1998	8	1. DRS (dry rolled sorghum); control (0%)
	8	2. DRS + 2.5% FA; supplemental fat (2.5%)
	8	3. SFS (steam flaked sorghum); control (0%)
	8	4. SFS + 2.5% FA; supplemental fat (2.5%)
	8	5. SFS + 5% FA; supplemental fat (5%)
Harvatine and Allen, 2006a,b,c	8	1. Control, cannulated cows; control (0%)
	8	2. SFA (saturated fatty acids), cannulated cows; supplemental fat (2.5%)
	8	3. Control, non-cannulated cows; control (0%)
	8	4. SFA (saturated fatty acids), non-cannulated cows; supplemental fat (2.5%)
Moallem et al., 2007a	14	1. Control; control (0%)
	14	2. PrFA:PrFA; supplemental fat (1.25%)
Moallem et al., 2007b	14	1. Control; control (0%)
	13	2. PrFA; supplemental fat (1.9%)
Relling and Reynolds, 2007	4	1. Control; control (0%)
	4	2. SFA (saturated fatty acids); supplemental fat (3.5%)
Thering et al., 2009	5	1. Control; control (0%)
	6	2. EB100 (Energy Booster 100); supplemental fat (3.5%)
Weiss & Pinos-Rodríguez, 2009	18	1. High forage - fat; control (0%)
	18	2. High forage + fat; supplemental fat (2.25%)
	18	3. Low forage - fat; control (0%)
	18	4. Low forage + fat; supplemental fat (2.25%)
Wang et al., 2010	16	1. SFA0 (saturated fatty acids 0%); control (0%)
	16	1. SFA1.5 (saturated fatty acids 1.5%); supplemental fat (1.5%)
	16	1. SFA3 (saturated fatty acids 3%); supplemental fat (3%)
Weiss et al., 2011	8	1. Control; control (0%)
	8	2. SFA (saturated fatty acids); supplemental fat (3%)
Bernard et al., 2012	16	1. Control; control (0%)
	16	2. SAT (saturated fat); supplemental fat (1.67%)
Greco et al., 2012	10	1. CTL; control (0%)
	10	2. SFA (saturated fatty acids); supplemental fat (1.7%)
Piantoni et al., 2015a,b	12	1. 20% fNDF + 0% SFFA; control (0%)
	12	2. 20% fNDF + 2% SFFA; supplemental fat (2%)
	12	3. 26% fNDF + 0% SFFA; control (0%)
	12	4. 26% fNDF + 2% SFFA; supplemental fat (2%)

Table 2. Estimated mean difference and 95% CI for dry matter and net energy intake, milk production, milk component concentration and yield, and feed efficiency in dairy cattle supplemented with a combination of C18:0 and C16:0 free fatty acids versus a no fat control.

			Parameter				[3]Heterogeneity	
Item	[1]N	[2]Supplemental fat (g/d)	Mean Difference	SE	*P* - value	95% CI	I^2	*P* - value
Intake								
DMI (kg/d)	40	632 ± 222.4	-0.06	0.181	0.7481	(-0.40, 0.28)	92.67	0.0001
NE_L intake (Mcal/d)	13	577 ± 249.8	2.13	0.617	0.0048	(0.79, 3.48)	97.41	0.0001
Milk Production								
Milk yield (kg/d)	39	596 ± 216.3	1.24	0.260	0.0001	(0.71, 1.76)	57.00	0.0001
3.5% FCM (kg/d)	21	631 ± 258.3	1.38	0.327	0.0004	(0.70, 2.06)	27.78	0.0699
Milk Composition								
Milk fat (%)	39	628 ± 227.5	0.08	0.028	0.0093	(0.02, 0.13)	55.51	0.0001
Milk protein (%)	39	645 ± 216.6	-0.02	0.017	0.3363	(-0.05, 0.02)	82.93	0.0001
Milk lactose (%)	23	551 ± 197.7	-0.04	0.021	0.0612	(-0.09, 0.00)	76.53	0.0001
Milk Component Yield								
Milk fat yield (kg/d)	38	645 ± 232.8	0.06	0.012	0.0001	(0.04, 0.09)	73.65	0.0001
Milk protein yield (kg/d)	38	624 ± 225.7	0.03	0.007	0.0008	(0.01, 0.04)	49.60	0.0044
Milk lactose yield (kg/d)	14	613 ± 150.5	0.05	0.031	0.1553	(-0.02, 0.11)	79.23	0.0002
Efficiency								
3.5% FCM/DMI (kg/kg per d)	11	524 ± 177.7	0.06	0.024	0.0244	(0.01, 0.12)	44.60	0.0462

[1] N = number of comparisons included in analysis.
[2] Average supplemental fat feeding rate (g/d) ± SD; weighted based on inverse variance of response variable.
[3] Heterogeneity indicates how much variation exists among treatment differences; I^2 estimates what proportion of total variation in mean differences is attributable to among-means variation.

The Benefits of Getting More Potassium into Lactating Cows

T. C. Jenkins[1], J. H. Harrison*, and G. J. Lascano
[1]Department of Animal & Veterinary Sciences, Clemson University
**Department of Animal Sciences, Washington State University*

Introduction

Potassium (**K**) is the principal intracellular cation of most body tissues. Potassium ions participate in many essential biological processes such as the maintenance of osmotic potential within cells, nerve impulse transmission, enzyme reactions in cellular metabolism, the maintenance of normal kidney function, and cardiac, skeletal and smooth muscle function. Because milk is an intracellular fluid, milk contains a large amount of K.

This paper reviews the responses of lactating dairy cows to increasing K concentration in the diet on milk yield and components. Because some K sources have had consistent positive effects on milk fat percentages, and milk fat percentages have been linked to the ruminal production of certain biohydrogenation intermediates, then data from several continuous culture experiments are reviewed to determine how K supplements affect biohydrogenation.

Negative K Balance in the Early Lactation Dairy Cow

Published research suggests that the early lactation dairy cow is in negative K balance (Bannink et al., 1999; Jarrett et al., 2012; Nennich et al., 2006; Silanikove et al., 1997). Potassium retention in this data set was positive for over 85% of cows in the calibration dataset; however, in a set of early lactation cows, K retention was negative for all cows (Nennich et al., 2006). Early lactation cows (less than 75 days in milk) had an average K retention of – 66 g/d (**Figure 1**). Excretion of K appears to be directly related to K intake. **Figure 2** shows the relationship of K intake and K excretion.

Potassium metabolism of cows in the early lactation dataset varied from cows in the calibration dataset. Early lactation cows tended to excrete greater amounts of K even though K intakes were similar to cows in the calibration dataset (Figure 2). Due to the greater K excretion and the greater secretion of K in milk, early lactation cows were in a negative K balance.

Potassium's role in milk production can be tied to the concept of dietary cation anion difference (**DCAD**). Potassium is a cation that raises the DCAD, which represents interaction among the macrominerals. Interacting effects among the macrominerals sodium (**Na**), K, chloride (**Cl**), and sulfur (**S**) have been observed in the pre-calving cow, but little has been written on this subject for the post-calving cow. DCAD affects the cow

[1] Contact at: Department of Animal & Veterinary Sciences, Clemson University, Clemson, SC 29630. E-mail: tjnkns@clemson.edu.

by altering its acid-base status. For a general review and broader examination of these and other related topics please see the review by Block (1994). There are differences in the response to DCAD that depends on the source of Na and K used in these studies. This difference appears to show up mainly in cows in the early lactation period.

Production Responses to K and DCAD

In 2012 we published a study that evaluated the relationship of level of K feeding in early lactation when DCAD was increased with K carbonate sesquihydrate (Harrison et al., 2012). Cows were on study from ~ 15 days in milk until ~ 85 days in milk. Diets were formulated to be similar in all nutrients except K (**Table 1**) with K levels of 1.3% and 2.1% of DM; and DCAD levels of 25 and 42 mEq/100 g of DM.

The inclusion of a higher amount of K in the early lactation diet resulted in an increase in production of milk, 3.5% fat-corrected milk, and milk fat (**Table 2**). This increase was not associated with an increase in dry matter intake (**DMI**), and therefore appears to be unrelated to energy intake.

Table 3 summarizes the evaluation we conducted on milk fat samples from this same study. Milk samples from one-half the cows in each treatment group that represented a range from low to high milk production were selected for characterization of milk fatty acids. A limited set of the milk fatty acids is shown in Table 3. The added dietary potassium carbonate decreased unsaturated and trans-fatty acids, and increased C18:0 in milk. This suggests that one mechanism for the increase in milk fat production is ruminally based.

Potassium and Heat Stress

With an increase in ambient temperature dairy cows rely on adaptive mechanisms to dissipate heat and these include: moving to shade if available, decreasing DMI, increasing water intake, and increasing evaporative loss via respiration and sweating. Mallonee et al. (1985) observed a 5 fold increase in loss of K via sweating when cows were provided shade during the hottest part of the day, 9.6 mg/m^2 vs 46.7 mg/m^2. When respiration is increased to dissipate heat, CO_2 is lost more quickly, plasma CO_2 partial pressure is lowered, and the pH of blood tends to rise. Potassium and Na are key to maintaining a blood acid-base balance, and their role is critical in times of heat stress and increased respiration rates.

Special Considerations

Our current dietary recommendations are to formulate for 1.6% K, and to increase to 1.8 to 2% for heat stress. Sodium levels can be increased to assist in achieving a DCAD of > 35 meq/100 g of DM. Sodium should not exceed 0.8% of the ration DM. There are three reasons that guidelines for Na and K are higher than NRC (2001). First, because early lactation cows eat less than mid-lactation cows, there is a need to increase nutrient concentrations to reflect reduced feed intakes. Second, most of the macro-

mineral research was conducted with low and medium producing cows; high producing cows secrete more of these minerals in milk and generate more acid in the rumen and blood. Third, the higher concentrations of Na and K represent an additional role these nutrients play in rumen buffering and acid-base balance, and recent data suggests that cows can be deficient in K and Na in early lactation.

No recommendation is given for Na because of its dependency on K and DCAD concentrations. Salt *per se* is not a required nutrient by dairy cows. However, because salt is one of the four taste sensors on the tongue we recommend a minimum of salt (~ 0.1 lb/d) in every lactation ration. Chloride should be kept to as close to the minimum NRC recommendations as possible to avoid complications due to chloride's contribution in subclinical metabolic acidosis.

Ruminal Explanation for K and Milk Fat

Increasing DCAD in diets fed to lactating cows has had positive effects on milk fat and milk fat yield. Iwaniuk and Erdman (2015) reported in a meta-analysis of 196 dietary treatments that milk fat percentage increased 0.1 for each increase in DCAD of 100 mEq/kg of DM. One explanation for the increase in milk fat percentage with increasing DCAD can be linked to ruminal fluid pH. Increasing DCAD was shown to increase ruminal fluid pH an average of 0.03-units per 100 mEq of DCAD/kg of dietary DM (Iwaniuk and Erdman, 2015). The increase in DCAD and ruminal fluid pH likely alters the types and amount of biohydrogenation (**BH**) intermediates produced by the rumen microbial population, which in turn increases milk fat. Therefore, the milk fat response to DCAD requires an understanding of 1) how BH intermediates are linked to milk fat synthesis and 2) how ruminal fluid pH is linked to the production of BH intermediates.

Biohydrogenation and Milk Fat Synthesis

Biohydrogenation of linoleic acid in the rumen begins with its conversion to conjugated linoleic acid (**CLA**). In this initial step, the number of double bonds remains the same but one of the double bonds is shifted to a new position by microbial enzymes. Normally, the double bonds in linoleic acid are separated by two single bonds, but in CLA, the double bonds are only separated by one single bond. Many types of CLA are produced in the rumen of dairy cows, but a common CLA produced from BH of linoleic acid is *cis*-9, *trans*-11 C18:2 (Jenkins et al., 2008). As BH progresses, double bonds in the CLA intermediates are then hydrogenated further to *trans* fatty acids having only one double bond. A final hydrogenation step by the ruminal microbes eliminates the last double bond yielding stearic acid as the final end product.

In cows on a typical forage diet, the major *trans* C18:1 produced in ruminal contents is *trans*-11 C18:1 (Zened et al., 2013). Most of the remaining isomers have double bonds distributed equally among carbons 9 through 16. The exact pathways for the production of these positional isomers are not known. Linoleic and linolenic acids are converted to several *trans* C18:1 and C18:2 intermediates during BH. Mosley et al. (2002) showed that the BH of oleic acid by mixed ruminal microorganisms involves the

formation of several positional isomers of *trans* C18:1 rather than only direct BH to form stearic acid as previously described.

Under certain dietary situations the rumen environment is altered and a portion of BH occurs via a pathway that produces *trans*-10, *cis*-12 CLA and *trans*-10 18:1. The *trans*-10, *cis*-12 CLA produced in the rumen travel via the blood to the mammary gland, where it inhibits the synthesis of milk fat by impairing the production of several enzymes essential for fat synthesis in the mammary gland (Jenkins and Harvatine, 2014). The *trans*-10, *cis*-12 CLA are also present in cows that produce acceptable milk fat levels, but at concentrations too low to cause milk fat depression (**MFD**).

The '*trans*-10 shift' in BH pathways is not a risk for MFD unless it is accompanied by a bottleneck at the terminal step of the pathway. Without a bottleneck, excess *trans*-10, *cis*-12 CLA is quickly and extensively converted to the *trans*-10 C18:1 intermediate, never accumulating to levels needed for MFD. With a bottle neck at the terminal step, there is excess accumulation of *trans*-10, *cis*-12 in the rumen leading to MFD. This can be seen by the associated increase in the *trans*-10 18:1 content of milk fat, which is indicative of the complex changes in ruminal BH pathways characteristic of MFD. Although *trans*-10 18:1 does not directly inhibit mammary synthesis of milk fat (Lock et al., 2007), it is relatively easy to analyze compared to *trans*-10, *cis*-12 CLA and other CLA isomers. Therefore, in general, this fatty acid can serve as a surrogate marker for the type of alterations in rumen BH that characterize diet-induced MFD.

The bottom line is that the type of feed the cow consumes affects rumen conditions, which in turn affects the amount and type of CLA produced. Since *trans*-10, *cis*-12 CLA overproduction in the rumen leads to MFD, excess *trans*-10, *cis*-12 CLA and therefore MFD can be controlled by paying close attention to several key nutritional risks.

Ruminal Fluid pH and Biohydrogenation Intermediates

Factors that can result in marked changes in ruminal fluid pH through any 24-h period include: dietary carbohydrate profile and rates of degradation of the carbohydrate fractions as affected by source, processing, and moisture; physically effective NDF (**peNDF**) supply as affected by source and particle size; and production of salivary buffers as a function of peNDF supply and source (Shaver, 2005). Despite our general understanding of these factors, the degree and duration of low ruminal fluid pH required to cause sufficient flux of unsaturated fatty acids through alternative pathways of ruminal BH is not known. Although data are limited, changes in ruminal fluid pH are most likely associated with MFD because they cause a change in the bacterial population favoring alternative BH pathways. Ruminal pH has independent effects on both extent of BH as well as on the profile of BH intermediates.

Martin and Jenkins (2002) examined the continuous culture incubations that were conducted at dilution rates of 0.05 and 0.10/h with pH values of 5.5 and 6.5, and 0.5 and 1.0 g/L of mixed soluble carbohydrate. They found that the most influential environmental factor on both extent of BH and *trans* FA profile was culture pH At pH

5.5, the concentration of *trans*-C18:1 and CLA were significantly reduced resulting from reduced extent of BH from linoleic acid. Similar effects were observed by Troegeler-Meynadier et al. (2003). Low amounts of CLA from reduced extent of BH at pH 6.0 could be due to low isomerase activity or to high reductase activity. Moreover, they found that low pH (pH 6.0) resulted in lower amount of *trans*-11 C18:1 at all incubation times compared with higher pH (pH 7.0), but concentration of *trans*-10 C18:1 were higher at 16 to 24 h of incubation indicating a shift in BH intermediates. Low pH inhibited initial isomerization and the second reduction (*trans*-11 C18:1 to stearic acid), leading to an accumulation of *trans*-11 C18:1 in ruminal cultures (Troegeler-Meynadier et al., 2006). Choi et al. (2005) reported that *cis*-9, *trans*-11 CLA are produced at pH higher than 6.2 by rumen bacteria, but *trans*-10, *cis*-12 CLA are produced more than *cis*-9 *trans*-11 CLA at lower pH. They concluded that *trans*-10, *cis*-12 CLA producing bacteria may be more aero and acid-tolerant than *cis*-9, *trans*-11 CLA producing bacteria.

Qiu et al. (2004) reported that reduced ruminal fluid pH can affect microbial populations, especially cellulolytic bacteria. Total cellulolytic bacteria numbers are reduced, accompanied by reduced acetate-to-propionate ratio and altered BH when pH was low. The ruminal fluid pH also influenced fungal growth and metabolism. Culturing ruminal fungi at pH 6.0 and pH 7.0 slowed BH compared with pH 6.5. CLA production was increased by pH 7.0 compared to pH 6.0 and pH 6.5. Therefore, optimum pH was 6.5 and 7.0 for BH and CLA production, respectively, by ruminal fungi (Nam and Garnsworthy, 2007).

Supplemental K Effects on Biohydrogenation Intermediates

Reports of increased milk fat yields following the addition of K to the diet raised questions if K altered ruminal BH and the type of CLA produced. A series of continuous culture experiments were run at Clemson University to determine if increasing K concentration in the culture contents was associated with a decline in the production of the *trans*-10, *cis*-12 isomer linked to MFD. The first experiment (Jenkins et al., 2014) consisted of four dosage levels of a 10% K_2CO_3 (w/w) stock solution (0, 10.6, 21.2, and 32 mL) injected directly into the fermenters twice daily immediately after each feeding (fermenters were fed 60 g of 1:1 forage to concentrate in two equal portions at 0800 and 1630 h). Distilled water was also injected (32, 21.4, 10.8, and 0 mL, respectively) to maintain a total injection (K_2CO_3 + water) volume of 32 mL/d. The K added was 0, 0.6, 1.2, and 1.8 g/d or 0 (K0), 1% (K1), 2% (K2), or 3% (K3) of the daily feed. Because aqueous solutions of K_2CO_3 are strongly alkaline, pH was expected to increase with increasing dosage of K_2CO_3. To determine if any changes in BH and fermentation could be attributed to effects on pH, a fifth treatment (NaOH) consisted of injection of sufficient 10% NaOH (w/w) each day to match the K3 pH.

As expected, pH averaged over the three sampling days increased ($P < 0.05$) linearly with increasing K, but remained in the 6.0 to 6.4 range (**Table 4**). Culture pH were similar for the K3 and NaOH treatments. Increasing K had effects on VFA proportions but not total VFA concentrations. As K addition to the cultures increased, there were linear decreases ($P < 0.05$) in propionate but increases ($P < 0.05$) in acetate

and acetate to propionate ratio. Addition of NaOH could not duplicate the VFA changes seen for K_2CO_3. K addition also affected the pattern of BH intermediates. As K addition increased, the daily production in mg/d of *trans*-11 18:1 and *cis*-9, *trans*-11 CLA both increased ($P < 0.05$) linearly. Conversely, K addition decreased ($P < 0.05$) *trans*-10 C18:1 but had no effect on *trans*-10, *cis*-12 CLA. The addition of K caused a shift in BH intermediates consistent with the improvement in milk fat % observed in previous lactation trials. Changes in BH intermediates also were caused by the NaOH treatment suggesting K might shift BH by elevating pH.

A second continuous culture experiment (Jenkins et al., 2014) was run to examine the effects of K in culture contents that had elevated *trans*-10, *cis*-12 CLA concentrations induced by feeding high fat. Six treatments were arranged as a 2 x 3 factorial with two levels of added soybean oil (0 and 4%) and 3 levels of added K (0, 1.5, and 3%). Potassium was introduced by injection of a 10% K_2CO_3 (w/w) stock solution (0, 16, and 32 ml/d) directly into the fermenters twice daily immediately after each feeding. Distilled water was also injected (32, 16, and 0 mL/d, respectively) to maintain a total injection (K_2CO_3 + water) volume of 32 mL/d. The K added was 0, 0.9, and 1.8 g/d or 0 (K0), 1.5% (K1.5), or 3% (K3) of the daily feed. Cultures on the low fat diet were fed 60 g of basal diet per day. Cultures on the high fat diet were fed 60 g of basal diet plus 2 g of soybean oil (mixed as a complete diet) for a total of 62 g of feed per day.

Similar to the first experiment, increasing K caused an increase ($P < 0.05$) in culture pH regardless of diet fat content (**Table 5**). Addition of K also affected VFA as in the first experiment, but differently depending on dietary fat content. For the low fat diet, increasing K again increased ($P < 0.05$) acetate and acetate to propionate ratio, and reduced ($P < 0.05$) propionate concentration. However, K had little effect on VFA when dietary fat content was high. As expected, the 4% added soybean oil increased ($P < 0.05$) *trans*-10, *cis*-12 CLA production from an average of 4.3 mg/d for the low fat diets to 53.8 mg/d for the high fat diets. Regardless of fat content in the diet, increasing K reduced ($P < 0.05$) *trans*-10, *cis*-12 CLA production supporting earlier results that K enhances milk fat content by re-directing the pathways of BH back to normal. As K decreased ($P < 0.01$) *trans*-10, *cis*-12 CLA, it also increased ($P < 0.05$) the production of *cis*-9, *trans*-11 CLA that is typical of normal BH.

Additional continuous culture experiments were run to determine if changes in BH intermediates seen for K_2CO_3 in the first two experiments could be duplicated with either KCl or with Na_2CO_3. Culture pH still increased ($P < 0.05$) from K_2CO_3 addition but not from KCl addition (**Table 6**). No changes in VFA, CLA, or *trans* monenes were observed following the addition of KCl. Carbonate effects on culture pH, VFA, and CLA were identical regardless if added as K_2CO_3 or as Na_2CO_3 (**Table 7**).

Conclusions

Early lactation cows can suffer from negative K balance due to greater K excretion, greater secretion of K in milk, and increased perspiration losses during heat stress.

With the inclusion of a higher amount of K in the early lactation diet, some studies showed an increase in production of milk, 3.5% FCM, and milk fat, which was not associated with an increase in DMI. The positive lactation responses to supplemental K supports the role of K ions in many essential biological processes such as the maintenance of osmotic potential within cells, nerve impulse transmission, enzyme reactions in cellular metabolism, the maintenance of normal kidney function, and cardiac, skeletal and smooth muscle function. Potassium supplementation also has increased milk fat percentages, which can be explained in part by reduced ruminal synthesis of biohydrogenation intermediates known to inhibit milk fat synthesis. The lowering of biohydrogenation intermediates that inhibit milk fat synthesis is likely mediated through the alkalizing effects of some K supplements to increase ruminal fluid pH.

References

Bannink, A., H. Valk, and A. M. Van Vuuren. 1999. Intake and excretion of sodium, potassium, and nitrogen and the effects on urine production by lactating dairy cows. J. Dairy Sci. 82:1008-1018.

Block E. 1994. Manipulation of dietary cation-anion difference on nutritionally related production diseases, productivity, and metabolic responses of dairy cows. J. Dairy Sci. 77:1437.

Choi, N. J., J. Y. Imm, S. J. Oh, B. C. Kim, H. J. Hwang, and Y. J. Kim. 2005. Effect of pH and oxygen on conjugated linoleic acid (CLA) production by mixed rumen bacteria from cows fed high concentrate and high forage diets. Anim. Feed Sci. Tech. 123-124:643-653.

Harrison, J.H., R. White, R.L. Kincaid, E. Block, T. Jenkins, and N. St Pierre. 2012. Effectiveness of potassium carbonate sesquihydrate to increase dietary cation-anion. J. Dairy Sci. 95:3919-3925.

Iwaniuk, M. E. and R. A. Erdman. 2015. Intake, milk production, ruminal, and feed efficiency responses to dietary cation-anion difference by lactating dairy cows. J. Dairy Sci. 98:8973-8985.

Jarrett, J., M. S. Taylor, T. Nennich, K. Knowlton, J. H. Harrison, and E. Block. 2012. Effect of stage of lactation and dietary calcium on potassium balance in lactating Holstein cows through twenty weeks of lactation. The Professional Animal Scientist 28:502-506.

Jenkins, T. C., and K. J. Harvatine. 2014. Lipids feeding and milk fat depression. Vet. Clin. Food Anim., pp 623-642, Elsevier Publishing.

Jenkins, T. C., R. J. Wallace, P. J. Moate, and E. E. Mosley. 2008. BOARD-INVITED REVIEW: Recent advances in biohydrogenation of unsaturated fatty acids within the rumen microbial ecosystem. J. Anim. Sci. 86:397-412.

Jenkins, T. C., W. C. Bridges, Jr., J. H. Harrison, and K. M. Young. 2014. Addition of potassium carbonate to continuous cultures of mixed ruminal bacteria shifts volatile fatty acids and daily production of biohydrogenation intermediates. J. Dairy Sci. 97:975-984.

Lock, A. L., C. Tyburczy, D. A. Dwyer, K. J. Harvatine, F. Destaillats, Z. Mouloungui, L. Candy, and D. E. Bauman. 2007. Trans-10 octadecenoic acid does not reduce milk fat synthesis in dairy cows. J. Nutr. 137:71-76.

Mallonee, P. G., D. K. Beede, R. J. Collier, and C. J. Wilcox. 1985. Production and physiological responses of dairy cows to varying dietary potassium during heat stress. J. Dairy Sci. 68:1479-1487.

Martin, S. A., and T. C. Jenkins. 2002. Factors affecting conjugated linoleic acid and trans-C18:1 fatty acid production by mixed ruminal bacteria. J. Anim. Sci. 80:3347-3352.

Mosley, E. E., G. L. Powell, M. B. Riley, and T. C. Jenkins. 2002. Microbial biohydrogenation of oleic acid to trans isomers in vitro. J. Lipid Res. 43:290-296.

Nam, I. S., and P. C. Garnsworthy. 2007. Factors influencing biohydrogenation and conjugated linoleic acid production by mixed rumen fungi. J. Microbiol. 45(3):199-204.

Nennich, T. D., J. H. Harrison, L. M. VanWieringen, N. R. St-Pierre, R. L. Kincaid, M. A. Wattiaux, D. L. Davidson, and E. Block. 2006. Prediction and evaluation of urine and urinary nitrogen and mineral excretion from dairy cattle. J. Dairy Sci. 89:353-364.

Qiu, X., M. L. Eastridge, K. E. Griswold, and J. L. Firkins. 2004. Effects of substrate, passage rate, and pH in continuous culture on flows of conjugated linoleic acid and trans-C18:1. J. Dairy Sci. 87:3473-3479.

Shaver, R. D. 2005. Feeding to minimize acidosis and laminitis in dairy cattle. Proc. Cornell Nutr. Conf. pp. 49-60.

Silanikove, N., E. Maltz, A. Halevi, and D. Shinder. 1997. Metabolism of water, sodium, potassium, and chlorine by high yielding dairy cows at the onset of lactation. J. Dairy Sci. 80:949-956.

Troegeler-Meynadier, A., L. Bret-Bennis, and F. Enjalbert. 2006. Rates and efficiencies of reactions of ruminal biohydrogenation of linoleic acid according to pH and polyunsaturated fatty acids concentrations. Reprod. Nutr. Dev. 46:713-724.

Troegeler-Meynadier, A., M. C. Nicot, C. Bayourthe, R. Moncoulon, and F. Enjalbert. 2003. Effects of pH and concentrations of linoleic and linolenic acids on extent and intermediates of ruminal biohydrogenation in vitro. J. Dairy Sci. 86:4054-4063.

Zened, A., F. Enjalbert, M.C. Nicot, and A. Troegeler-Meynadier. 2013. Starch plus sunflower oil addition to the diet of dry dairy cows results in a trans-11 to trans-10 shift of biohydrogenation. J. Dairy Sci. 96:451-459.

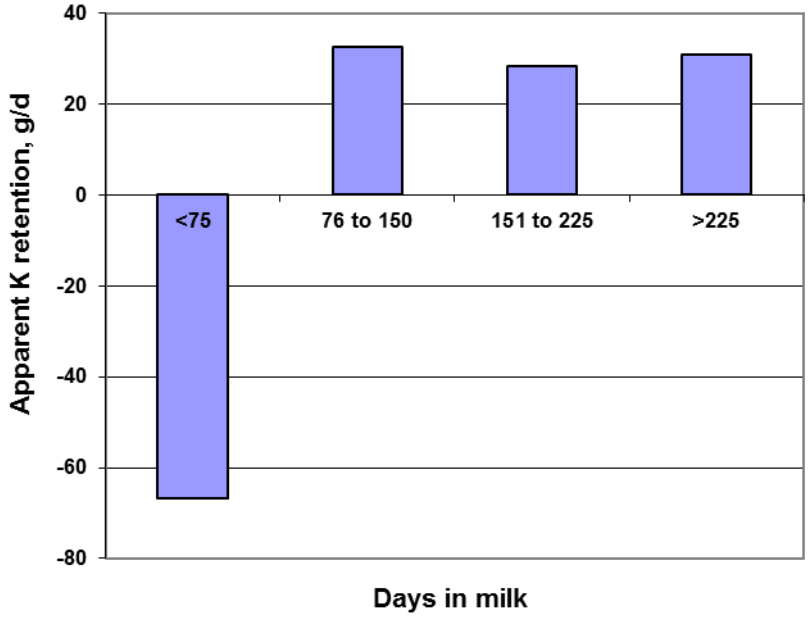


Figure 1. Apparent potassium retention of lactating cows at various days in milk.

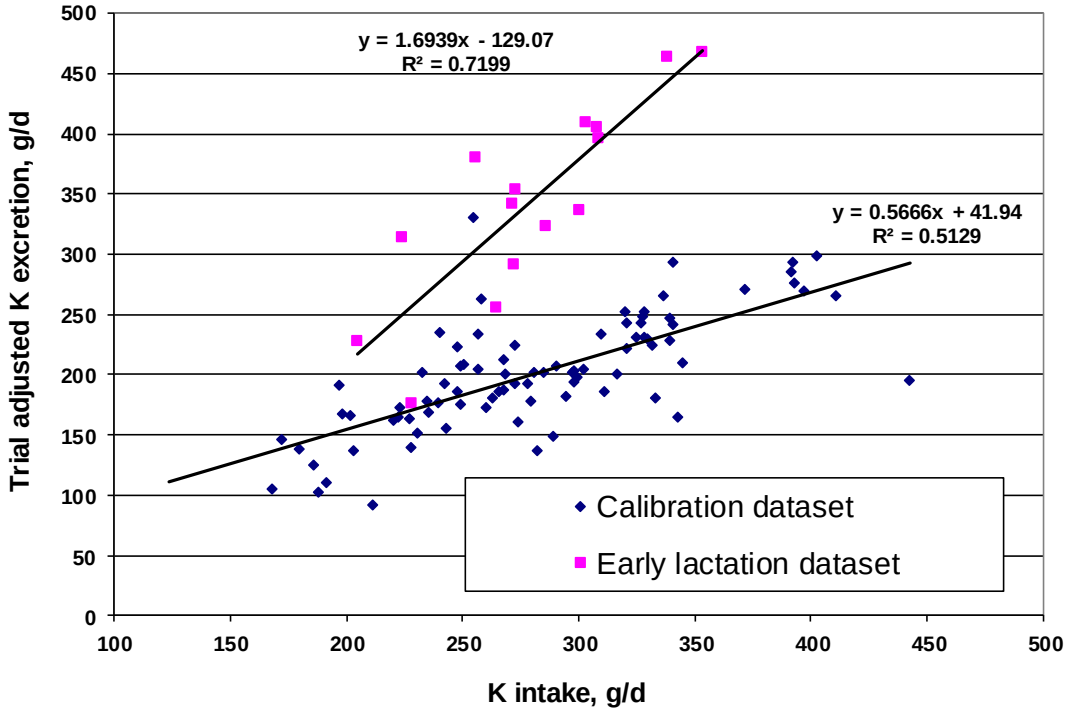


Figure 2. The relationship of potassium intake and potassium excretion for cows in the calibration and early lactation datasets.

Table 1. Summary of nutrient composition of diets in early lactation DCAD study.

% of DM	Control	DCAD+
DM	60.1	59.4
CP	16.1	16.1
ADF	19.8	19.3
NDF	35.0	34.7
Ash	7.0	8.6
Ca	0.69	0.66
P	0.37	0.36
Mg	0.43	0.45
K	1.28	2.07
DCAD[1]	32	53

[1] DCAD (mEq/100 g of DM) = (Na + K) – (Cl + S).

Table 2. Body weight, dry matter intake, milk production, and milk component production in early lactation DCAD study.

Item	Control	DCAD+	*P* <
BW, kg	669	674	0.49
DMI, kg/d	26.2	26.8	0.20
Milk, kg/d	39.3	40.8	0.01
ECM, kg/d	41.3	44.3	0.24
3.5% FCM, kg/d	42.2	46.1	0.09
Fat, kg/d	1.55	1.75	0.03
True protein, kg/d	1.16	1.14	0.12

Table 3. Milk fatty acid composition in early lactation DCAD study.

Item, % of total FA	**DCAD**	**Con**	***P* <**
C16:1	1.32	1.47	0.03
C18:0	14.2	12.6	0.02
t6,t8 C18:1	0.31	0.36	0.03
t9 C18:1	0.26	0.29	0.07
t10 C18:1	0.4	0.68	0.03
t11 C18:1	1.05	1.43	0.11
t12 C18:1	0.55	0.61	0.09
c9, t11 CLA	0.34	0.44	0.03

Table 4. Changes in pH, VFA, and biohydrogenation intermediates in continuous cultures dosed with increasing amounts of K_2CO_3.

	Treatment[1]					
	K0	K1	K2	K3	NaOH	SE
pH d8-10[a]	6.01	6.22	6.25	6.38	6.29	0.12
VFA, mol/100 mol						
Acetate[ab]	48.2	48.7	52.0	52.1	48.7	1.0
Propionate[ab]	36.2	35.6	32.2	32.9	36.7	1.4
Ac/Pr[ab]	1.34	1.37	1.66	1.60	1.33	0.09
Total VFA, mM	103.5	95.2	98.4	95.1	95.4	6.0
BH intermediates, mg/d						
t10 C18:1	537.6	499.8	461.1	538.2	575.8	38.4
t10, c12 CLA	11.6	11.3	7.9	7.6	13.2	2.6
c9, t11 CLA[a]	2.3	4.9	7.1	6.8	6.8	1.1

[a] Linear response of K0 through K3 ($P < 0.05$).
[b] K3 and NaOH differ ($P < 0.05$).
[1] K_2CO_3 injected into culture flasks to provide the equivalent of 0, 1, 2, and 3% added K. The NaOH treatment used injections of NaOH into fermentation flasks to maintain the same pH as the K3 treatment.

Table 5. Changes in pH, VFA, and biohydrogenation intermediates in continuous cultures fed a low or high fat diet in combination with three concentrations of added K_2CO_3.

	0% Fat			4% Fat			
	0	1.5	3	0	1.5	3	SEM
pH d8-10 [ab]	5.99	6.32	6.36	5.91	6.13	6.17	0.10
VFA, mol/100 mol							
Acetate [bc]	46.6	56.1	57.2	50.7	52.1	50.3	2.5
Propionate [abc]	34.7	25.8	22.4	33.5	31.3	32.0	2.0
Ac/Pr [abc]	1.35	2.21	2.59	1.58	1.72	1.60	0.19
Total VFA, mM	76.7	69.7	71.6	79.9	85.8	79.1	7.1
BH intermediates, mg/d							
trans-18:1[abc]	320.9	140.0	132.3	883.9	773.7	444.7	69.0
t10, c12 CLA [ab]	6.9	3.4	2.7	65.8	44.7	50.9	3.6
c9, t11 CLA [b]	2.6	5.7	7.0	2.7	6.4	8.3	1.0

[a] Fat effect ($P < 0.05$).
[b] K effect ($P < 0.05$).
[c] Fat x K interaction ($P < 0.05$).

Table 6. Changes in pH, VFA, and biohydrogenation intermediates in continuous cultures fed a low or high fat diet in combination with two sources of added K.

	0% Fat			3% Fat			
	0% K	K_2CO_3	KCl	0% K	K_2CO_3	KCl	SE
pH d10 [b]	6.36^{x}	6.57^{y}	6.35^{x}	6.33^{x}	6.47^{y}	6.21^{y}	0.071
4 hVFA, mol/100 mol							
Acetate [ab]	51.8^{y}	53.9^{x}	50.3^{y}	49.3^{y}	52.6^{x}	51.1^{y}	0.89
Propionate [ab]	30.2^{x}	26.3^{y}	30.6^{x}	32.0^{x}	28.8^{y}	31.4^{x}	1.10
Ac/Pr [ab]	1.72^{y}	2.06^{x}	1.68^{y}	1.56^{y}	1.83^{x}	1.60^{y}	0.062
Total VFA, mM	76.2^{xy}	67.3^{y}	79.0^{x}	83.7^{x}	67.4^{y}	79.0^{x}	6.29
BH intermediates, mg/d							
t10-18:1[ab]	25.6	17.8	24.3	221.2^{x}	143.6^{y}	196.5^{x}	15.9
t11-18:1 [abc]	69.4	104.5	65.4	130.8^{y}	272.3^{x}	148.6^{y}	16.9
t10, c12 CLA [a]	2.14	2.21	2.08	37.3	36.4	41.7	2.34
c9, t11 CLA [abc]	3.09	5.80	3.60	7.25^{y}	16.00^{x}	8.97^{y}	1.00

[a] Fat effect ($P < 0.05$).
[b] K effect ($P < 0.05$).
[c] Fat x K interaction ($P < 0.05$).
[xy] Means within a fat level with the same letter were not different ($P < 0.05$).

Table 7. Changes in pH, VFA, and biohydrogenation intermediates in continuous cultures fed a low or high fat diet in combination with two sources of added K.

	Treatment				
	CON	MIX	KCO3	NaCO3	SE
pH [a]	6.05	6.40	6.31	6.36	0.11
VFA, mol/100 mol					
Acetate [a]	58.85	64.57	65.50	66.57	2.62
Propionate [a]	27.24	23.00	22.70	21.23	1.57
Ac/Pr [a]	2.12	2.89	2.93	3.15	0.42
BH intermediates, mg/d					
t-10 18:1[a]	504.1	256.2	232.8	266.0	32.8
t-12 18:1[a]	7.66	0.46	0.92	3.70	1.88
c9, t11 CLA [a]	8.37	11.07	11.39	12.57	1.19
t10, c12 CLA [b]	19.73	12.10	12.38	13.97	3.64

[1]1:1 mix of K carbonate and Na carbonate
[a] CON differed from others ($P < 0.05$).
[b] CON differed from others ($P < 0.10$).

Pre- and Postpartum Nutritional Management to Optimize Energy Balance and Fertility in Dairy Cows

***Felipe Cardoso*[1]**
Department of Animal Sciences, University of Illinois

Introduction

During the transition period from late gestation through early lactation, the dairy cow undergoes tremendous metabolic adaptations (Bell, 1995). The endocrine changes during the transition period are necessary to prepare the dairy cow for parturition and lactogenesis. As peak milk yield increases, the transition period for dairy cows becomes much more challenging with most infectious diseases and metabolic disorders occurring during this time (Drackley, 1999; Grummer, 1995). Decreased dry matter intake (**DMI**) during late gestation influences metabolism leading to fat mobilization from adipose tissue and glycogen from liver.

Nutrient demand for milk synthesis is increased in early lactation; if no compensatory intake of nutrients is achieved to cope with the requirement, reproductive functions (i.e., synthesis and secretion of hormones, follicle ovulation, and embryo development) may be depressed. Milk production increases faster than energy intake in the first 4 to 6 weeks after calving, and thus high yielding cows will experience negative energy balance (**NEB**). Nutritional strategies and feeding management during pre-calving and post-calving periods impact health, productivity, and fertility of high producing dairy cows. Formulating diets to meet requirements of the cows while avoiding over-consumption of energy, may improve outcomes of the transition period and lead to improved fertility. Management to improve cow comfort and ensure good intake of the ration is pivotal for success. Impacts of the transition program should be evaluated in a holistic way that considers disease occurrence, productivity, and fertility.

Studies over the last 2 decades clearly established the link between nutrition and fertility in ruminants (Robinson et al., 2006; Wiltbank et al., 2006; Grummer et al., 2010; Santos et al., 2010; Cardoso et al., 2013; Drackley and Cardoso, 2014). Dietary changes can cause an immediate and rapid alteration in a range of humoral factors that can alter endocrine and metabolic signaling pathways crucial for reproductive function (Boland et al., 2001; Diskin et al., 2003). Moreover, periconceptional nutritional environment in humans and other animals is critical for the long-term setting of postnatal phenotype (Fleming et al., 2015). Restricting the supply of B-vitamins and methionine during the periconceptional period in sheep resulted in adverse cardiometabolic health in postnatal offspring (Sinclair et al., 2007). Feeding female mice a low-protein diet during the preimplantation period of pregnancy resulted in a reduction in amino acid (**AA**) concentration in uterine fluid and serum and attendant changes in

[1] Contact: 290 Animal Sciences Laboratory, 1207 W. Gregory, Urbana Illinois 61801. (217) 300-2303. Email: cardoso2@illinois.edu

the AA profile of the blastocyst (Eckert et al., 2012).

Strategies have been used to improve the reproductive performance of dairy cows through alteration of nutritional status (Santos et al., 2008; Santos et al., 2001). In other species, dietary supplementation with specific AA (e.g., arginine, glutamine, leucine, glycine, and methionine) had beneficial effects on embryonic and fetal survival and growth through regulation of key signaling and metabolic pathways (Del Curto et al., 2013; Wang et al., 2012). Methionine is the most limiting AA in lactating cows (NRC, 2001), but supplementation of diets with crystalline methionine has been excluded because free methionine is quickly and almost totally degraded by the microorganisms in the rumen (NRC, 2001). In contrast, supplementing rumen-protected methionine (**RPM**) has a positive effect on milk protein synthesis in dairy cows (Pisulewski et al., 1996; Ordway, 2009; Osorio et al., 2013). Although the role of methionine in bovine embryonic development is unknown, there is evidence that methionine availability alters the transcriptome of bovine preimplantation embryos *in vivo* (Penagaricano et al., 2013) and its contents (Acosta et al., 2016).

Reproduction, Nutrition, and Health

A widespread assumption is that fertility of modern dairy cows is decreasing, particularly for Holstein-Friesian genetics, at least in part because of unintended consequences of continued selection for high milk production. This assumption has been challenged recently (LeBlanc, 2010; Bello et al., 2012). There is a wide distribution of reproductive success both within and among herds. For example, within five California herds encompassing 6,396 cows, cows in the lowest quartile for milk yield in the first 90 days postpartum (32.1 kg/day) were less likely to have resumed estrous cycles by 65 days postpartum than cows in quartiles two (39.1 kg/day), three (43.6 kg/day), or four (50.0 kg/day); milk production did not affect risk for pregnancy (Santos et al., 2009). Changes in management systems and inadequacies in management may be more limiting for fertility of modern dairy cows than their genetics per se.

Dairy cows are susceptible to production disorders and diseases during the peripartal period and early lactation, including milk fever, ketosis, fatty liver, retained placenta, displaced abomasum, metritis, mastitis, and lameness (Mulligan et al., 2006; Ingvartsen and Moyes, 2013; Roche et al., 2013). There is little evidence that milk yield per se contributes to greater disease occurrence. However, peak disease incidence (shortly after parturition) corresponds with the time of greatest NEB, the peak in blood concentrations of nonesterified fatty acids (**NEFA**), and the greatest acceleration of milk yield (Ingvartsen et al., 2003). Peak milk yield occurs several weeks later. Disorders associated with postpartum NEB also are related to impaired reproductive performance, including fatty liver (Rukkwamsuk et al., 1999; Jorritsma et al., 2003) and ketosis (Walsh et al., 2007; McArt et al., 2012). Cows that lost > 1 body condition score (BCS) unit (1-5 scale) had greater incidence of metritis, retained placenta, and metabolic disorders (displaced abomasum, milk fever, ketosis) as well as a longer interval to first breeding than cows that lost < 1 BCS unit during the transition (Kim and Suh, 2003).

Indicators of NEB are highly correlated with lost milk production, increased disease, and decreased fertility (Ospina et al., 2010; Chapinal et al., 2012). However, the extent to which NEB is causative for peripartal health problems rather than just a correlated phenomenon must be examined critically (Roche et al., 2013). For example, in transition cows inflammatory responses may decrease DMI, cause alterations in metabolism, and predispose cows to greater NEB or increased disease (Bertoni et al., 2008; Graugnard et al., 2012 and 2013; Ingvartsen and Moyes, 2013). Inducing a degree of calculated NEB in mid-lactation cows similar to what periparturient cows often encounter does not result in marked increases in ketogenesis or other processes associated with peripartal disease (Moyes et al., 2009). Nevertheless, early postpartal increases in NEFA and decreases in glucose concentrations were strongly associated with pregnancy at first insemination in a timed artificial insemination (TAI) program (Garverick et al., 2013). Although concentrations of NEFA and glucose were not different between cows that ovulated or did not before TAI, probability of pregnancy decreased with greater NEFA and increased with greater glucose concentrations at day 3 postpartum (Garverick et al., 2013). In support of these findings, early occurrence of subclinical ketosis is more likely to decrease milk yield and compromise fertility. McArt et al. (2012) found that cows with subclinical ketosis detected between 3 to 7 days after calving were 0.7 times as likely to conceive to first service and 4.5 times more likely to be removed from the herd within the first 30 days in milk compared with cows that developed ketosis at 8 days or later.

Cows that successfully adapt to lactation (Jorritsma et al., 2003) and can avoid metabolic (Ingvartsen et al., 2003) or physiological imbalance (Ingvartsen and Moyes, 2013) are able to support both high milk production and successful reproduction while remaining healthy. Decreased fertility in the face of increasing milk production may be attributable to greater severity of postpartal NEB resulting from inadequate transition management or increased rates of disease. Competition for nutrients between the divergent outcomes of early lactation and subsequent pregnancy will delay reproductive function. Because NEB interrupts reproduction in most species, including humans, inappropriate nutritional management may predispose cows to both metabolic disturbances and impaired reproduction. Cows must make “metabolic decisions” about where to direct scarce resources, and in early lactation nutrients will be directed to milk production rather than to the next pregnancy (Friggens, 2003).

Different nutritional strategies have been proposed to improve reproduction of the dairy cow with no detrimental effect on lactation performance. Feeding high quality forages, controlled-energy (**CE**) diets, or adding supplemental fat to diets are some of the most common ways to improve energy intake in cows (Cardoso et al., 2013; Drackley and Cardoso, 2014; Mann et al., 2015). Reproduction of dairy cattle may be benefited by maximizing DMI during the transition period, minimizing the incidence of periparturient problems (Cardoso et al., 2013; Drackley and Cardoso, 2014).

Prepartum Dietary Considerations

Our research group has shown that controlling energy intake during the dry period to near calculated requirements leads to better transition success (Grum et al., 1996; Dann et al., 2005 and 2006; Douglas et al., 2006; Janovick et al., 2011; Graugnard et al., 2012 and 2013; Ji et al., 2012). Our research drew from earlier reports that limiting nutrient intakes to requirements of the cows was preferable to over-consumption of energy (e.g., Kunz et al., 1985). Cows fed even moderate-energy diets (1.50 to 1.60 Mcal of NE_L/kg of DM) will easily consume 40 to 80% more NEL than required during both far-off and close-up periods (Dann et al., 2005 and 2006; Douglas et al., 2006; Janovick and Drackley, 2010). Cows in these studies were all less than 3.5 BCS (1-5 scale) at dry-off, and were fed individually TMR based on corn silage, alfalfa silage, and alfalfa hay with some concentrate supplementation. We have no evidence that the extra energy and nutrient intake was beneficial in any way. More importantly, our data indicate that allowing cows to over-consume energy even to this degree may predispose them to health problems during the transition period if they face stressors or challenges that limit DMI (Cardoso et al., 2013).

Our studies indicate that prolonged over-consumption of energy during the dry period can decrease post-calving DMI (Douglas et al., 2006; Dann et al., 2006; Janovick and Drackley, 2010). Over-consuming energy results in negative responses of metabolic indicators, such as higher NEFA and beta-hydroxybutyrate (**BHB**) in blood and more triacylglycerol (**TAG**) in the liver after calving (Douglas et al., 2006; Janovick et al., 2011). Alterations in cellular and gene-level responses in liver (Loor et al., 2006 and 2007) and adipose tissue (Ji et al., 2012) potentially explain many of the changes at the cow level. Over-consumption of energy during the close-up period increases the enzymatic “machinery” in adipose tissue for TAG mobilization after calving, with transcriptional changes leading to decreased lipogenesis, increased lipolysis and decreased ability of insulin to inhibit lipolysis (Ji et al., 2012). Controlling energy intake during the dry period also improved neutrophil function postpartum (Graugnard et al., 2012) and so may lead to better immune function.

Our data demonstrate that allowing dry cows to consume more energy than required, even if cows do not become noticeably over-conditioned, results in responses that would be typical of overly fat cows. Because energy that cows consume in excess of their requirements must either be dissipated as heat or stored as fat, we speculated that the excess is accumulated preferentially in internal adipose tissue depots in some cows. Moderate over-consumption of energy by non-lactating cows for 57 days led to greater deposition of fat in abdominal adipose tissues (omental, mesenteric, and perirenal) than in cows fed a high-bulk diet to control energy intake to near requirements (Drackley et al., 2014). The NEFA and signaling molecules released by visceral adipose tissues travel directly to the liver, which may cause fatty liver, subclinical ketosis, and secondary problems with liver function.

Data from our studies support field observations that controlled-energy dry cow programs decrease health problems (Beever, 2006). Other research groups

(Rukkwamsuk et al., 1998; Holcomb et al., 2001; Holtenius et al., 2003; Vickers et al., 2013) have reached similar conclusions about controlling energy intake during the dry period, although not all studies have shown benefits (Winkleman et al., 2008). Application of these principles can be through controlled limit-feeding of moderate energy diets or ad libitum feeding of high-bulk, low-energy rations (Janovick and Drackley, 2010; Janovick et al., 2011; Ji et al., 2012) as proposed by others (Beever, 2006).

Nutritionally complete diets must be fed and the TMR must be processed appropriately so that cows do not sort the bulkier ingredients (Janovick and Drackley, 2010). Feeding bulky forage separately from a partial TMR or improper forage processing will lead to variable intake among cows, with some consuming too much energy and some too little. Underfeeding relative to requirements, where nutrient balance also is likely limiting, leads to increased incidence of retained placenta and metritis (Mulligan et al., 2006). Merely adding a quantity of straw to a diet is not the key principle; rather, the diet must be formulated to limit the intake of energy (approximately 1.3 Mcal of NE_L/kg of DM to limit intake to about 15 Mcal/day for typical Holstein cows) but meet the requirements for protein, minerals and vitamins. Reports of increased transition health problems or poor reproductive success (Whitaker et al., 1993) with “low energy” dry cow diets must be examined carefully to discern whether nutrient intakes were adequate.

Fresh Cow (postpartum) Dietary Considerations

Less is known about diet formulation for the immediate postpartum period to optimize transition success and subsequent reproduction. Increased research is needed in this area. Proper dietary formulation during the dry period or close-up period will maintain or enable ruminal adaptation to higher grain diets after calving. Failure to do so may compromise early lactation productivity. For example, Silva-del-Rio et al. (2010) attempted to duplicate the dietary strategy of Dann et al. (2006) by feeding either a low-energy far-off diet for 5 weeks followed by a higher-energy diet for the last 3 weeks before parturition, or by feeding the higher-energy diet for the entire 8-week dry period. They found that cows fed the higher-energy diet for only 3 weeks before parturition produced less milk than cows fed the diet for 8 weeks (43.8 vs. 48.5 kg/day). However, the far-off dry period diet contained 55.1% alfalfa silage and 38.5% wheat straw but no corn silage. In comparison, the higher-energy dry period diet and the early lactation diet both contained 35% corn silage. Ruminal adaptation likely was insufficient for cows fed the higher energy diet for only 3 weeks.

A major area of concern in the fresh cow period is the sudden increase in dietary energy density leading to subacute ruminal acidosis (SARA) which can decrease DMI and digestibility of nutrients (Mulligan and Doherty, 2008). Adequate physical form of the diet, derived either from ingredients or mixing strategy, must be present to stimulate ruminal activity and chewing behavior (Zebeli and Metzler-Zebeli, 2012), although good methods to quantify “adequacy” remain elusive. Dietary starch content and fermentability likely interact with forage characteristics and ration physical form. Dann

and Nelson (2011) compared three dietary starch contents (primarily from corn starch) in the fresh cow period for cows fed a CE-type ration in the dry period. Milk production was greatest when starch content was moderate (23.2% of DM) or low (21.0% of DM) in the fresh cow diet compared with high (25.5% of DM). If SARA decreases DMI and nutrient availability to the cow, NEFA mobilization and increased ketogenesis may follow. In addition, rapid starch fermentation in the presence of NEFA mobilization leads to bursts of propionate reaching the liver, which may decrease feeding activity and DMI according to the hepatic oxidation theory (Allen et al., 2009). A moderate starch content (ca. 23-25% of DM) with starch of moderate fermentability (for example, ground dry corn rather than high-moisture corn or ground barley) along with adequate effective forage fiber may be the best strategy for fresh cows. Recent research also has demonstrated that high grain diets can lead to greater numbers of gram-negative bacteria such as *E. coli* with resulting increases in endotoxin present in the rumen, which may decrease barrier function and inflammatory responses in the cow (Zebeli and Metzler-Zebeli, 2012).

Supplemental fats have been widely investigated as a way to increase dietary energy intake and improve reproduction (Thatcher et al., 2011). A novel strategy to use polyunsaturated fatty acid (PUFA) supplements to improve reproduction has been reported (Silvestre et al., 2011). Cows fed calcium salts of safflower oil from 30 days before to 30 days after calving, followed by calcium salts of fish oil to 160 days postpartum, had greater pregnancy rates and higher milk production. The mechanism is believed to be provision of greater amounts of linoleic acid (omega-6 PUFA) until early postpartum which improves uterine health, followed by greater amounts of omega-3 PUFA from fish oil to decrease early embryonic loss (Thatcher et al., 2011). The negative effects of turbulent transitions on reproduction are established early postpartum, likely during the first 10 days to 2 weeks postpartum (Butler, 2003; McArt et al., 2012; Garverick et al., 2013). By 8 weeks postpartum, >95% of cows should be at or above energy balance (Sutter and Beever, 2000). Use of targeted prepartum and postpartum strategies may minimize health problems and lessen NEB and thereby improve subsequent fertility.

Body Condition Score

The role of excessive BCS in contributing to transition problems and impaired subsequent reproduction is well established and has been discussed by many authors (Drackley et al., 2005; Garnsworthy et al., 2008; Roche et al., 2013). Cows with excessive body lipid reserves mobilize more of that lipid around calving, have poorer appetites and DMI before and after calving, have impaired immune function, have increased indicators of inflammation in blood, and may be more subjected to oxidative stress (Contreras and Sordillo, 2011). What constitutes “excessive” BCS relative to the cow’s biological target remains controversial. Garnsworthy (2007) argued that the average optimal BCS has decreased over time with increased genetic selection for milk yield, perhaps related to correlated changes in body protein metabolism (**Figure 1**). Recommendations for optimal BCS at calving have trended downward over the last two decades and, in the author’s opinion, a score of about 3.0 (1-5 scale) represents a good

goal at present. Adjustment of average BCS should be a longstanding project and should not be undertaken during the dry period.

Our group showed that cows fed high-energy (1.58 Mcal of NE_L/kg of DM) diets during the last 4 weeks before calving lost more BCS in the first 6 weeks postpartum than those fed controlled energy (1.32 Mcal of NE_L/kg of DM) diets (−0.43 and −0.30, respectively) (Cardoso et al., 2013). The effect of BCS change on cow's fertility is clear. Carvalho et al. (2014) showed that cows that either gained or maintained BCS from calving to 21 days after calving had higher (38.2 and 83.5%, respectively) pregnancy per AI at 40 days than cows that lost BCS (25.1%) during that same period. Previously, Santos et al. (2009) had shown that cows that had > 1.0 BCS unit change from calving to AI at approximately 70 days postpartum had lower pregnancy per AI (28%) than cows that lost < 1.0 BCS unit (37.3%) or did not have a BCS change (41.6%). In a grazing system, researchers from New Zealand suggested that BCS at calving should be targeted at 2.75-3.0 to optimize production, while reducing liver lipid accumulation and the negative effects of inflammation on liver function (Roche et al., 2013; Akbar et al., 2015).

The Importance of Amino Acids

Some AA are limiting for optimal milk production as evidenced by an increase in milk yield, percentage of milk protein, and milk protein yield after supplementation with specific, rumen-protected AA. The first three limiting AA for milk production are considered to be methionine, lysine (NRC, 2001), and histidine (Huhtanen et al., 2002). In addition, many AA can have positive effects on physiological processes that are independent of their effects on synthesis of proteins (Wu, 2013). Fertilization and the first few days of embryo development occur in the oviduct. By about 5 days after estrus, the embryo arrives in the uterine horn. The embryo reaches the blastocyst stage by 6 to 7 days after estrus. The embryo hatches from the zona pellucida by about 9 days after estrus and then elongates between days 14 to 19. The elongating embryo secretes the protein interferon-tau that is essential for rescue of the corpus luteum and continuation of the pregnancy. By days 25 to 28 the embryo attaches to the caruncles of the uterus and begins to establish a vascular relationship with the dam through the placenta. During all the time prior to embryo attachment, the embryo is free-floating and is dependent upon uterine secretions for energy and the building blocks for development, including AA. Thus, it is critical to understand the changes in AA concentrations in the uterus that accompany these different stages of embryo development.

The lipid profile of oocytes and the early embryo can be influenced by the environment of the cow. Our group ran a trial with the objective to determine the effect of supplementing rumen-protected methionine on DNA methylation and lipid accumulation in preimplantation embryos of dairy cows (Acosta et al., 2016). Lactating Holsteins entering their 2nd or greater lactation were randomly assigned to one of two treatments from 30 ± 2 DIM to 72 ± 2 DIM: Control (CON; n = 5, fed a basal diet with a 3.4:1 Lys:Met) and Methionine (MET; n = 5, fed the basal diet plus Smartamine M to a 2.9:1 Lys:Met). Embryos were flushed 6.5 d after artificial insemination. Embryos with

stage of development of 4 or greater were used for analysis. For lipids, fluorescence intensity of Nile Red staining was compared against a negative control embryo (subtraction of background). A total of 37 embryos were harvested from cows (MET = 16; CON = 21). Cows receiving MET had greater lipid accumulation (7.3 arbitrary units) when compared with cows receiving CON (3.7 arbitrary units). There were no treatment effects on number of cells or stage of development. In conclusion, cows supplemented with methionine produced embryos with higher lipid concentration when compared to CON which could potentially serve as an important source of energy for the early developing embryo.

The requirements for complete development of bovine embryos have not yet been determined. Current culture conditions allow development of bovine embryos to the blastocyst stage (day 7-8) and even allow hatching of a percentage of embryos (day 9); however, conditions have not been developed in vitro that allow elongation of embryos. The methionine requirements for cultured pre-implantation bovine embryos (day 7-8) was determined in studies from the University of Florida (Bonilla et al., 2010). There was a surprisingly low methionine requirement (7 µM) for development of embryos to the blastocyst stage by day 7. However, development to the advanced blastocyst stage by day 7 appeared to be optimized at around 21 µM (Bonilla et al., 2010). Thus, the results of these studies indicate that development of morphologically normal bovine embryos did not require elevated methionine concentrations (> 21 µM), at least during the first week after fertilization.

Researchers at the University of Wisconsin (Toledo et al., 2015) conducted a trial with a total of 309 cows (138 primiparous and 171 multiparous) that were blocked by parity and randomly assigned to two treatments: 1) CON - cows fed a ration formulated to deliver 2,500 g of MP with 6.9% Lys (% of MP) and 1.9% Met (% of MP) and 2) RPM - cows fed a ration formulated to deliver 2,500 g of MP with 6.9% Lys (% of MP) and 2.3% Met (% of MP). Cows were randomly assigned to three pens with head-locks and fed a single basal TMR twice daily. From 28 to 128 DIM, after the AM milking, cows were head-locked for 30 minutes and the TMR of CON and RPM cows were individually top-dressed with 50 g of DDG or 50 g of a mix of DDG (29 g) and Smartamine M (21 g), respectively. Following a double ovsynch protocol, cows were inseminated and pregnancy checked at 28 (plasma Pregnancy Specific Protein-B concentration) and at 32, 47, and 61 d (ultrasound). Individual milk samples were taken once a month and analyzed for composition. There were no statistical differences in milk production but RPM cows had a higher milk protein concentration. Cows fed the methionine-enriched diet had a lower pregnancy loss from 21 to 61 d after AI (16.7% RPM cows vs. 10.0% CON cows). Pregnancy losses between days 28 and 61 were not different in the primiparous cows (12.8% CON and 14.6% RPM); however, pregnancy losses between treatments were significant for the multiparous cows (19.6% CON vs. 6.1% RPM).

Conclusions

Formulation and delivery of appropriate diets that limit total energy intake to requirements but also provide proper intakes of all other nutrients before calving can help lessen the extent of NEB after calving. Effects of such diets on indicators of

metabolic health are generally positive, suggesting the potential to lessen effects of periparturient disease on fertility. Supplementation of cows with methionine during the final stages of follicular development and early embryo development, until Day 7 after breeding, lead to lipid accumulation changes in the embryos and resulted in differences in gene expression in the embryo. Methionine supplementation seems to impact the preimplantation embryo in a way that enhances its capacity for survival because there is strong evidence that endogenous lipid reserves serve as an energy substrate. The lower pregnancy losses from cows fed a methionine enriched diets suggest that methionine favors the embryo survival, at least in multiparous cows.

References

Acosta, D. A. V., A.C. Denicol, P. Trbulo, M.I. Rivelli, C. Skenandore, Z. Zhou, D. Luchini, M.N. Corrêa, P.J. Hansen, F.C. Cardoso. 2016. Effects of rumen-protected methionine and choline supplementation on the preimplantation embryo in Holstein cows. Theriogenology; 85:1669-1679.

Akbar, H., T. M. Grala, M. Vailati Riboni, F. C. Cardoso, G. Verkerk, J. McGowan, K. Macdonald, J. Webster, K. Schutz, S. Meier, L. Matthews, J. R. Roche, and J. J. Loor. 2015. Body condition score at calving affects systemic and hepatic transcriptome indicators of inflammation and nutrient metabolism in grazing dairy cows. J. Dairy Sci. 98:1019–1032.

Beever, D. E. 2006. The impact of controlled nutrition during the dry period on dairy cow health, fertility and performance. Animal Reproduction Science. 96:212-226.

Bell, A. W. 1995. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. J. Anim. Sci. 73:2804-2819.

Bello, N. M., J. S. Stevenson, and R. J. Tempelman. 2012. Invited review: Milk production and reproductive performance: Modern interdisciplinary insights into an enduring axiom. J. Dairy Sci. 95:5461-5475.

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

Boland, M. P., P. Lonergan, and D. O'Callaghan. 2001. Effect of nutrition on endocrine parameters, ovarian physiology, and oocyte and embryo development. Theriogenology. 55:1323–40.

Bonilla, L, D. Luchini, E. Devillard, and P. J. Hansen. 2010. Methionine requirements for the preimplantation bovine embryo. J. Reprod. Dev. 56:527–32.

Butler, W. R. 2003. Energy balance relationships with follicular development, ovulation and fertility in postpartum dairy cows. Livestock Prod. Sci. 83:211.

Cardoso, F. C., S. J. LeBlanc, M. R. Murphy, and J. K. Drackley. 2013. Prepartum nutritional strategy affects reproductive performance in dairy cows. J. Dairy Sci. 96:5859–71.

Carvalho, P.D., A.H. Souza, M. C. Amundson, K. S. Hackbart, M. J. Fuenzalida, M. M. Herlihy, H. Ayres, A. R. Dresch, L. M. Vieira, J. N. Guenther, R. R. Grummer, P. M. Fricke, R. D. Shaver, and M. C. Wiltbank. 2014. Relationships between fertility and postpartum changes in body condition and body weight in lactating dairy cows. J. Dairy Sci. 97:3666–3671.

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

Contreras, G. A. and L. M. Sordillo. 2011. Lipid mobilization and inflammatory responses during the transition period of dairy cows. Comparative Immunology, Microbiology and Infectious Diseases. 34:281–289.

Dann, H. M., D. E. Morin, M. R. Murphy, G. A. Bollero, and J. K. Drackley. 2005. Prepartum intake, postpartum induction of ketosis, and periparturient disorders affect the metabolic status of dairy cows. J. Dairy Sci. 88:3249-3264.

Dann, H. M., N. B. Litherland, J. P. Underwood, M. Bionaz, A. D'Angelo, J. W. McFadden, and J. K. Drackley. 2006. Diets during far-off and close-up dry periods affect periparturient metabolism and lactation in multiparous cows. J J. Dairy Sci. 89: 3563-3577.

Dann, H. M. and B. H. Nelson. 2011. Early lactation diets for dairy cattle – focus on starch. In Proceedings of the 2011 Cornell Nutrition Conference for Feed Manufacturers, Syracuse, NY, USA.

DelCurto, H, G. Wu, and M. C. Satterfield. 2013. Nutrition and reproduction: links to epigenetics and metabolic syndrome in offspring. Curr. Opin. Clin. Nutr. Metab. Care.16:385–91.

Diskin, M. G., D. R. Mackey, J. F. Roche, J. M. Sreenan. 2003. Effects of nutrition and metabolic status on circulating hormones and ovarian follicle development in cattle. Anim. Reprod. Sci. 78:345–70.

Douglas, G. N., T. R. Overton, H. G. Bateman, H. M. Dann, and J. K. Drackley. 2006. Prepartal plane of nutrition, regardless of dietary energy source, affects periparturient metabolism and dry matter intake in Holstein cows. J. Dairy Sci. 89:2141-2157.

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

Drackley, J. K., H. M. Dann, G. N. Douglas, N. A. Janovick Guretzky, N. B. Litherland, J. P. Underwood, and J. J. Loor. 2005. Physiological and pathological adaptations in dairy cows that may increase susceptibility to periparturient diseases and disorders. Italian Journal of Animal Sci. 4:323-344.

Drackley, J. K. and F. C. Cardoso. 2014. Prepartum and postpartum nutritional management to optimize fertility in high-yielding dairy cows in confined TMR systems. Animal. 8:S1, 5-14.

Drackley, J. K., R. L. Wallace, D. Graugnard, J. Vasquez, B. F. Richards, and J. J. Loor. 2014. Visceral adipose tissue mass in non-lactating dairy cows fed diets differing in energy density. J. Dairy Sci. 97:3420-3430.

Eckert, J. J., R. Porter, A. J. Watkins, E. Burt, S. Brooks, H. J. Leese, P. G. Humpherson, I. T. Cameron, and T. P. Fleming. 2012. Metabolic induction and early responses of mouse blastocyst developmental programming following maternal low protein diet affecting life-long health. PLoS One; 7:e52791.

Fleming, T. P., M. A. Velazquez, J. J. Eckert. D. Embryos, and D. Barker. 2015. J. Dev. Orig. Health Dis. 6:377–83.

Friggens, N. C. 2003. Body lipid reserves and reproductive cycle: towards a better understanding. Livestock Production Sci. 83:219-236.
Garnsworthy, P. C. 2007. Body condition score in dairy cows: targets for production and fertility. In Recent advances in animal nutrition – 2006 (ed. PC Garnsworthy and J Wiseman), pp. 61-86. Nottingham University Press, Nottingham, UK.
Garnsworthy, P. C., K. D. Sinclair, and R. Webb. 2008. Integration of physiological mechanisms that influence fertility in dairy cows. Animal. 2:1144-1152.
Garverick, H. A., M. N. Harris, R. Vogel-Bluel, J. D. Sampson, J. Bader, W. R. Lamberson, J. N. Spain, M. C. Lucy, and R. S. Youngquist. 2013. Concentrations of nonesterified fatty acids and glucose in blood of periparturient dairy cows are indicative of pregnancy success at first insemination. J. Dairy Sci. 96:181-188.
Graugnard, D. E., M. Bionaz, E. Trevisi, K. M. Moyes, J. L. Salak-Johnson, R. L. Wallace, J. K. Drackley, G. Bertoni, and J. J. Loor. 2012. Blood immunometabolic indices and polymorphonuclear leukocyt e function in peripartum dairy cows are altered by level of dietary energy prepartum. J. Dairy Sci. 95:1749-1758.
Graugnard, D. E., K. M. Moyes, E. Trevisi, M. J. Khan, D. Keisler, J. K. Drackley, . Bertoni, and J. J. Loor. 2013. Liver lipid content and inflammometabolic indices in peripartal dairy cows are altered in response to prepartal energy intake and postpartal intramammary inflammatory challenge. J. Dairy Sci. 96:918-935.
Grum, D. E., J. K. Drackley, R. S. Younker, D. W. LaCount, and J. J. Veenhuizen. 1996. Nutrition during the dry period and hepatic lipid metabolism of periparturient dairy cows. J. Dairy Sci. 79:1850-1864.
Grummer, R. R. 1995. Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. J. Anim. Sci. 73:2820-2833.
Grummer, R. R., M. C. Wiltbank, P. M. Fricke, R. D. Watters, and N. Silva-Del-Rio. 2010. Management of dry and transition cows to improve energy balance and reproduction. J Reprod. Dev; 56(Suppl):S22–8.
Holcomb, C. S., H. H. Van Horn, H. H. Head, M. B. Hall, and C. J. Wilcox. 2001. Effects of prepartum dry matter intake and forage percentage on postpartum performance of lactating dairy cows. J. Dairy Sci. 84:2051-2058.
Holtenius, K., S. Agenäs, C. Delavaud, and Y. Chilliard. 2003. Effects of feeding intensity during the dry period. 2. Metabolic and hormonal responses. J. Dairy Sci. 86:883-891.
Huhtanen, P., V. Vanhatalo, and T. Varvikko. 2002. Effects of abomasal infusions of histidine, glucose, and leucine on milk production and plasma metabolites of dairy cows fed grass silage diets. J. Dairy Sci. 85:204-216.
Ingvartsen, K. L., R. J. Dewhurst, and N. C. Friggens. 2003. On the relationship between lactational performance and health: is it yield or metabolic imbalance that cause production diseases in dairy cattle? A position paper. Livestock Production Sci. 83:277–308.
Ingvartsen, K. L. and K. Moyes. 2013. Nutrition, immune function and health of dairy cattle. Animal 7 (Suppl. 1), 112-122.
Janovick, N. A. and J. K. Drackley. 2010. Prepartum dietary management of energy intake affects postpartum intake and lactation performance by primiparous and multiparous Holstein cows. J. Dairy Sci. 93:3086–3102.

Janovick, N. A., Y. R. Boisclair, and J. K. Drackley. 2011. Prepartum dietary energy intake affects metabolism and health during the periparturient period in primiparous and multiparous Holstein cows. J. Dairy Sci. 94:1385-1400.
Ji, P., J. S. Osorio, J. K. Drackley, and J. J. Loor. 2012. Overfeeding a moderate energy diet prepartum does not impair bovine subcutaneous adipose tissue insulin transduction and induces marked changes in peripartal gene network expression. J. Dairy Sci. 95:4333-4351.
Jorritsma, R., T. Wensing, T. A. M. Kruip, T. Vos PLAM, and J. P. T. M. Noordhuizen. 2003. Metabolic changes in early lactation and impaired reproductive performance in dairy cows. Veterinary Research. 34:11-26.
Kim, I. H. and G. H. Suh. 2003. Effect of the amount of body condition loss from the dry to near calving periods on the subsequent body condition change, occurrence of postpartum diseases, metabolic parameters and reproductive performance in Holstein dairy cows. Theriogenology. 60:1445-1456.
Kunz, P. L., J. W. Blum, I. C. Hart, J. Bickel, and J. Landis. 1985. Effects of different energy intakes before and after calving on food intake, performance and blood hormones and metabolites in dairy cows. Animal Production. 40:219-231.
LeBlanc, S. J. 2010. Assessing the association of the level of milk production with reproductive performance in dairy cattle. Journal of Reproduction and Development 56(Suppl.), S1-S7.
Loor, J. J., H. M. Dann, N. A. Janovick-Guretzky, R. E. Everts, R. Oliveira, C. A. Green, N. B. Litherland, S. L. Rodriguez-Zas, H. A. Lewin, and J. K. Drackley. 2006. Plane of nutrition prepartum alters hepatic gene expression and function in dairy cows as assessed by longitudinal transcript and metabolic profiling. Physiological Genomics. 27:29-41.
Loor, J. J., R. E. Everts, M. Bionaz, H. M. Dann, D. E. Morin, R. Oliveira, S. L. Rodriguez-Zas, J. K. Drackley, and H. A. Lewin. 2007. Nutrition-induced ketosis alters metabolic and signaling gene networks in liver of periparturient dairy cows. Physiological Genomics. 32:105-116.
McArt, J. A., D. V. Nydam, and G. R. Oetzel. 2012. Epidemiology of subclinical ketosis in early lactation dairy cattle. J. Dairy Sci. 95:5056-5066.
Mann, S., F. A. Leal Yepes, T. R. Overton, J. J. Wakshlag, A. L. Lock, C. M. Ryan, D. V. Nydam. 2015. Dry period plane of energy: Effects on feed intake, energy balance, milk production, and composition in transition dairy cows. J. Dairy Sci. 98:3366–3382.
Moyes, K. M., J. K. Drackley, J. L. Salak-Johnson, D. E. Morin, J. C. Hope, and J. J. Loor. 2009. Dietary-induced negative energy balance has minimal effects on innate immunity during a Streptococcus uberis mastitis challenge in dairy cows during mid-lactation. J. Dairy Sci. 92:4301-4316.
Mulligan, F. J., L. O'Grady, D. A. Rice, and M. L. Doherty. 2006. A herd health approach to dairy cow nutrition and production diseases of the transition cow. Animal Reproduction Sci. 96:331-353.
Mulligan, F. J. and M. L. Doherty. 2008. Production diseases of the transition cow. Veterinary Journal. 176:3-9.
NRC. 2001. Nutrient Requirements of Dairy Cattle. Seventh revised edition. Washington, DC: Natl. Acad. Press.

Ordway, R. S., S. E. Boucher, N. L. Whitehouse, C. G. Schwab, and B. K. Sloan. 2009. Effects of providing two forms of supplemental methionine to periparturient Holstein dairy cows on feed intake and lactational performance. J Dairy Sci. 92:5154–66.
Osorio, J. S., P. Ji, J. K. Drackley, D. Luchini, and J. J. Loor. 2013. Supplemental Smartamine M or MetaSmart during the transition period benefits postpartal cow performance and blood neutrophil function. J Dairy Sci. 96:6248–63.
Ospina, P. A., D. V. Nydam, T. Stokol, and T. R. Overton. 2010. Association between the proportion of sampled transition cows with increased nonesterified fatty acids and β-hydroxybutyrate and disease incidence, pregnancy rate, and milk production at the herd level. J. Dairy Sci. 93:3595-3601.
Penagaricano, F., A. H. Souza, P. D. Carvalho, A. M. Driver, R. Gambra, J. Kropp, K. S. Hackbart, D. Luchini, R. D. Shaver, M. C. Wiltbank, and H. Khatib. 2013. Effect of maternal methionine supplementation on the transcriptome of bovine preimplantation embryos. PLoS One. 8:e72302.
Pisulewski, P. M., H. Rulquin, J. L. Peyraud, R. Verite. 1996. Lactational and systemic responses of dairy cows to postruminal infusions of increasing amounts of methionine. J. Dairy Sci. 79:1781–91.
Robinson, J. J., C. J. Ashworth, J. A. Rooke, L. M. Mitchell, and T. G. McEvoy. 2006. Nutrition and fertility in ruminant livestock. Anim. Feed Sci. Technol. 126:259–76.
Roche, J. R., A. W. Bell, T. R. Overton, and J. J. Loor. 2013. Nutritional management of the transition cow in the 21st century – a paradigm shift in thinking. Animal Production Sci. 53:1000-1023.
Rukkwamsuk, T, T. Wensing, and M. J. Geelen. 1998. Effect of overfeeding during the dry period on regulation of adipose tissue metabolism in dairy cows during the periparturient period. J. Dairy Sci. 81:2904-2911.
Rukkwamsuk, T, T. Wensing, and T. A. M. Kruip. 1999. Relationship between triacylglycerol concentration in the liver and first ovulation in postpartum dairy cows. Theriogenology. 51:1133-1142.
Santos, J. E., E. J. DePeters, P. W. Jardon, J. T. Huber. 2001. Effect of prepartum dietary protein level on performance of primigravid and multiparous Holstein dairy cows. J. Dairy Sci. 84:213–24.
Santos, J. E., R. L. Cerri, and R. Sartori. 2008. Nutritional management of the donor cow. Theriogenology. 69:88–97.
Santos, J. E., H. M. Rutigliano, and M. F. Sa Filho. 2009. Risk factors for resumption of postpartum estrous cycles and embryonic survival in lactating dairy cows. Animal Reproduction Sci. 110:207-221.
Santos, J. E., R. S. Bisinotto, E. S. Ribeiro, F. S. Lima, L. F. Greco, C. R. Staples, and W. W. Thactcher. 2010. Applying nutrition and physiology to improve reproduction in dairy cattle. Soc. Reprod. Fertil. Suppl. 67:387–403.
Silva-del-Rio, N, P. M. Fricke, and R. R. Grummer. 2010. Effects of twin pregnancy and dry period strategy on milk production, energy balance, and metabolic profile in dairy cows. J. Animal Sci. 88:1048-1060.
Silvestre, F. T., T. S. M. Carvalho, N. Francisco, J. E. P. Santos, C. R. Staples, T. C. Jenkins, and W. W. Thatcher. 2011. Effects of differential supplementation of fatty acids during the peripartum and breeding periods of Holstein cows: I. Uterine and metabolic responses, reproduction, and lactation. J. Dairy Sci. 94:189-204.

Sinclair, K. D., C. Allegrucci, R. Singh, D. S. Gardner, S. Sebastian, J. Bispham, A. Thurston, J. F. Huntley, W. D. Rees, C. A. Maloney, R. G. Lea, J. Craigon, T. G. McEvoy, and L. E. Young. 2007. DNA methylation, insulin resistance, and blood pressure in offspring determined by maternal periconceptional B vitamin and methionine status. Proc. Natl. Acad. Sci U S A. 104: 19351–6.

Sutter, F. and D. E. Beever. 2000. Energy and nitrogen metabolism in Holstein-Friesen cows during early lactation. Animal Sci. 70:503-514.

Thatcher, W. W., J. E. P. Santos, and C. R. Staples. 2011. Dietary manipulations to improve embryonic survival in cattle. Theriogenology. 76:1619-1631.

Toledo, M., G.M. Baez, E. Trevisol, N. E. Lobos, A. Garcia-Guerra, J. N. Guen, D. Luchini, R. D. Shaver, M. C. Wiltbank. 2015. Effect of top-dressing rumen-protected methionine in lactating Holstein cows II: Fertility and embryo development. J. Dairy Sci. Vol. 98, Suppl. 2. Page 301.

Vickers, L. A., D. M. Weary, D. M. Veira, and M. A. G. von Keyserlingk. 2013. Feeding a higher forage diet prepartum decreases incidences of subclinical ketosis in transition dairy cows. J. Animal Sci. 91:886-894.

Walsh, R. B., J. S. Walton, D. F. Kelton, S. J. LeBlanc, K. E. Leslie, and T. F. Duffield. 2007. The effect of subclinical ketosis in early lactation on reproductive performance of postpartum dairy cows. J. Dairy Sci. 90:2788-2796.

Wang, J, Z. Wu, D. Li, N. Li, S. V. Dindot, M. C. Satterfield, F. W. Bazer, and G. Wu. 2012. Nutrition, epigenetics, and metabolic syndrome. Antioxid Redox Signal; 17:282–301.

Whitaker, D. A., E. J. Smith, G. O. da Rosa, and J. M. Kelly. 1993. Some effects of nutrition and management on the fertility of dairy cattle. Veterinary Record. 133:61-64.

Wiltbank, M. C., H. Lopez, R. Sartori, S. Sangsritavong, and A. Gumen. 2006. Changes in reproductive physiology of lactating dairy cows due to elevated steroid metabolism. Theriogenology. 65:17–29.

Winkleman, L. A., T. H. Elsasser, and C. K. Reynolds. 2008. Limit-feeding a high-energy diet to meet energy requirements in the dry period alters plasma metabolite concentrations but does not affect intake or milk production in early lactation. J. Dairy Sci. 91:1067-1079.

Wu, G, F. W. Bazer, M. C. Satterfield, X. Li, X. Wang, G. A. Johnson, R. C. Burghardt, Z. Dai, J. Wang, and Z. Wu. 2013. Impacts of arginine nutrition on embryonic and fetal development in mammals. Amino Acids. 45:241-256.

Zebeli, Q. and B. U. Metzler-Zebeli. 2012. Interplay between rumen digestive disorders and diet-induced inflammation. Research in Veterinary Sci. 93:1099-1108.

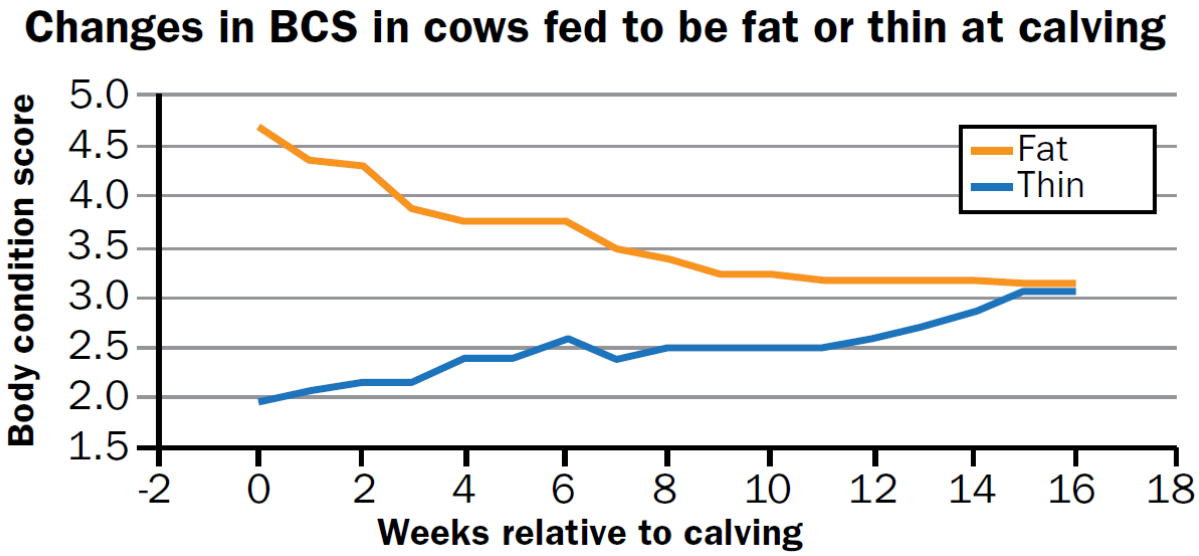


Figure 1. Changes in BCS in cows fed to be fat or thin at calving.

Update on B Vitamins for Lactating Dairy Cows

Christiane L. Girard[1] and Mélissa Duplessis
Agriculture and Agri-Food Canada
Sherbrooke Research and Development Centre

Introduction

For B vitamins as for all other nutrients, the ideal situation is when the supply is equal to the needs. On the one hand, there is deficiency as soon as the supply is lower than the needs; even sub-clinical deficiency has a metabolic cost because to survive, the cells have to use alternate, less efficient metabolic pathways. On the other hand, if the supply is greater than the needs, then you have losses in feces and urine and there is also a metabolic cost to dispose of surpluses. Dairy nutritionists balance rations in order to meet this ideal situation for major nutrients but B vitamins are seldom taken into account. But what do we know about B-vitamin needs and supply in dairy cows?

Estimating the Needs or the Requirements?

In humans, B-vitamin requirements are defined as the amount needed to sustain good health. In a high producing dairy cow, two supplementary components have to be taken into account: the objective to maximize metabolic efficiency and, for some B vitamins, the heavy drain imposed by their secretion in colostrum and milk. For example, concentrations of folates and vitamin B_{12} were 6 and 9 times greater in colostrum than in milk 39 days after calving, respectively (Duplessis et al., 2016). In humans and non-ruminants, estimation of the minimum requirement, i.e. the lowest intake to support normal function, is essential to define a dietary recommendation for a specific nutrient. The first step to quantify the minimum requirement is to identify a marker, often the activity of an enzyme or the vitamin concentration in a specific tissue, which will respond early to a lack of the studied vitamin. The second step is to feed a basal diet deficient only in this vitamin and supplemented with increasing doses of this nutrient in order to obtain a dose-response curve for the chosen marker (Combs, 2012). Obviously this approach is not working in ruminants because even when feeding a diet deficient in B vitamins, an unknown but not negligible amount of B vitamins synthesized in the rumen are available for the cow (Bechdel et al., 1928).

Consequently, in dairy cows as opposed to non-ruminants, B-vitamin needs and requirements are not the same. The need is the amount of vitamin requested by the tissues to maintain an optimal metabolic activity whereas the requirement is the amount to include in the diet to reach this objective. This difference between need and requirement is the amount of B vitamins synthesized by the ruminal microflora (Bechdel et al., 1928), generally in amounts sufficient to avoid apparition of deficiency symptoms. This situation probably explains why there were so few attempts to define dairy cow

[1] Contact: 2000 College Street, Sherbrooke, Quebec J1M 0C8, Telephone: 819-780-7233, Email: christiane.girard@agr.gc.ca

requirements. Nevertheless, there is evidence in the literature that dairy cows could benefit from B-vitamin supplements. Although these results do not allow one to quantify a requirement, they indicate that the supply does not always equate the needs. There is no or only very limited information on the effects of thiamin, riboflavin, pantothenic acid, and vitamin B_6 supplements on production and metabolic activity of dairy cows. Consequently, only results from experiments using niacin, biotin, folic acid, and vitamin B_{12} supplements are briefly described in this section.

Niacin. The name "niacin" is used for two active molecules, nicotinic acid and nicotinamide. Niacin is the essential component of nicotinamide adenine dinucleotide (**NAD**) and nicotinamide adenine dinucleotide phosphate (**NADP**) involved with more than 100 enzymes in all oxidation-reduction reactions. Niacin does not completely fit the definition of a vitamin because the molecule is synthesized from the amino acid tryptophan, although the importance of endogenous synthesis of niacin differs greatly among species (Combs, 2012). In preruminant calves, endogenous synthesis of niacin is sufficient to avoid apparition of deficiency symptoms if the diet provides a sufficient amount of tryptophan (Hoppner and Johnson, 1955). Interestingly, endogenous synthesis of niacin from tryptophan is suppressed in rats by ketone bodies (Shastri et al., 1968) and fatty liver (Fukuwatari and Shibata, 2013). Nevertheless, the importance of the tryptophan-niacin pathway for dairy cows across the gestation-lactation cycle is still unknown.

Nicotinic acid supplements reduce lipolysis in normal and ketotic cows (Waterman and Schultz, 1972; Waterman et al., 1972). Decreased plasma concentrations of non-esterified fatty acids and β-hydroxybutyrate and increased plasma glucose are the most frequently reported responses during use of nicotinic acid supplements, although the response is highly variable among experiments (Schwab et al., 2005; Niehoff et al., 2009; Yuan et al., 2012; Pires et al., 2016). Moreover, in *in vitro* and *in vivo* experiments, niacin supplements are frequently reported to increase the number of protozoa as well as microbial protein synthesis (Schussler et al., 1978; Riddell et al., 1980; Riddell et al., 1981; Dennis et al., 1982; Shields et al., 1983; Brent and Bartley, 1984; Horner et al., 1988a and b; Erickson et al., 1990; Ottou and Doreau, 1996; Aschemann et al., 2012; Niehoff et al., 2013). These effects on ruminal fermentation and lipolysis led to numerous experiments on the effects of niacin supplements on cow metabolism and production performance. According to a meta-analysis (Schwab et al., 2005) using data from 27 studies published between 1980 and 1998, a dietary supplement of 6 g of nicotinic acid per day had no effect on lactation performance of dairy cows. However, at a dose of 12 g/d, supplementary nicotinic acid increased fat yield and tended to increase 3.5% fat-corrected milk and protein yield. As there was no effect of the vitamin supplement on dry matter intake, feed efficiency tended also to be increased. Use of supplementary nicotinic acid has been also studied for its pharmacological effects on vasodilation to alleviate the consequences of heat-stress on lactating dairy cows. For production and metabolic responses, the responses differs among experiments (Di Costanzo et al., 1997; Wrinkle et al., 2012; Zimbelman et al., 2010 and 2013; Lohölter et al., 2013; Rungruang et al., 2014; Pineda et al., 2016).

Biotin. Biotin is likely to be of great importance in ruminants because it is a coenzyme for two essential enzymes for gluconeogenesis and it is involved in regulation of gene expression of many enzymes critical for glucose metabolism. In addition, biotin plays key roles in lipid and amino acid metabolism. Two meta-analyses on the effects of dietary supplements of biotin on milk production and composition of Holstein dairy cows were published in 2011 (Chen et al., 2011; Lean and Rabiee, 2011). Each of them used data from 11 comparisons with only 6 comparisons shared by both studies; the biotin supplement dose was generally 20 mg/d and exclusion and inclusion criteria differed between these studies. In spite of these differences, the conclusions were similar (**Table 1**). Such results illustrate that for dairy cows, biotin supply is frequently lower than the need although they partially hide the variability among experiments. Ferreira and collaborators (2007) stressed that supplementary biotin was more likely to increase milk and milk component yields in high-producing cows than in low-producing ones because the metabolic demand was greater in the former. However, in some experiments even high-producing cows did not respond to biotin supplementation (Rosendo et al., 2004). Biotin supplements at doses varying from 10 to 20 mg/d frequently improved hoof health.

Folic acid. The term “folic acid” is used either as the generic name of the vitamin or specifically, for the synthetic form of the vitamin. The term “folates” applies to the numerous biologically active forms. Folates have the single biochemical function of accepting and releasing one-carbon units for DNA synthesis and replication and thus, cell division. Folate coenzymes also provide one-carbon units for *de novo* formation of methyl groups essential to, for example, DNA methylation (which controls gene transcription and genetic stability) and synthesis of phosphatidylcholine, choline, creatine, and many neurotransmitters. Folic acid supplements, given orally or by intramuscular injections, increased milk production and milk protein yield during the first part of the lactation in multiparous cows (Girard and Matte, 1998; Graulet et al., 2007; Girard et al., 2009, Li et al., 2016). Except for one (Li et al., 2016), none of these experiments observed an increase in dry matter intake suggesting that supplementary folic acid increased efficiency of protein metabolism. Moreover, folate metabolism in mammary epithelial cells seems to be a critical regulatory point for synthesis of milk protein in many species, including dairy cows (Menzies et al., 2009). The absence of effects of the folic acid supplements on lactation performance observed in some experiments could be due to a low vitamin B_{12} supply (Girard et al., 2005; Preynat et al., 2009a).

Vitamin B_{12}. Vitamin B_{12} acts as a coenzyme in only two metabolic reactions. The vitamin is a coenzyme for methionine synthase; this interface between folic acid and vitamin B_{12} metabolism is so critical that a lack of vitamin B_{12} causes a secondary folate deficiency, even in presence of a sufficient folic acid supply (Scott, 1999). Besides this role, the other vitamin B_{12}-dependent enzyme, methylmalonyl-coenzyme A mutase, plays a major role in ruminants for the entry of propionate in the Krebs cycle and gluconeogenesis (McDowell, 2000).

Vitamin B_{12} is synthesized by ruminal bacteria if the cobalt supply is sufficient (Martens et al., 2002). Incidentally, it has been observed that, in spite of a sufficient dietary cobalt supply in dairy cows, the lowest plasma concentrations of vitamin B_{12} are observed during the first weeks of lactation (Elliot et al., 1965; Mykkänen and Korpela, 1981; Girard and Matte, 1999; Girard et al., 2005; Kincaid and Socha, 2007). Nevertheless, oral or parenteral supplements of vitamin B_{12} generally fail to affect milk and milk component yields in cows (Frobish and Davis, 1977; Croom et al., 1981; Graulet et al., 2007; Akins et al., 2013). However, as compared to a supplement of folic acid alone, a combined supplement of vitamin B_{12} and folic acid given to primiparous cows during the first weeks of lactation increased energy-corrected milk, packed cell volume and blood hemoglobin and decreased serum methylmalonic acid concentrations (Girard and Matte, 2005). The effect on blood hemoglobin and packed cell volume suggests that a low vitamin B_{12} supply interferes with folate metabolism decreasing DNA synthesis and blood red cell formation (Bills et al., 1992). Accumulation of methylmalonic acid in serum indicates that a low vitamin B_{12} supply also affects the other vitamin B_{12}-dependent enzyme, essential to propionate utilization. These observations support the hypothesis that a suboptimal vitamin B_{12} supply, especially during early lactation may limit the effects of folic acid supplements. Indeed, a combined supplement of folic acid and vitamin B_{12} has been reported to improve metabolic efficiency, especially energy metabolism (Graulet et al., 2007; Preynat et al., 2009b; Gagnon et al., 2015; Duplessis et al., 2014a). Moreover, possibly through an improvement of the energy balance in early lactation, the combined supplement of vitamins changes the expression of genes involved in differentiation of ovarian follicles (Gagnon et al., 2015), increases the number of large follicles and the size of the dominant follicle (Ghaemialehashemi, 2013) and decreases the interval between calving and the first insemination (Duplessis et al., 2014b). Nevertheless, production and metabolic responses to a combined supplement of folic acid and vitamin B_{12} are variable as illustrated in **Table 2**.

In the 5 experiments described in Table 2, multiparous dairy cows received by intramuscular injections a combined supplement of folic acid and vitamin B_{12} during the 3 to 4 weeks before the expected calving date and in early lactation. Dry matter intake and milk production of control cows were similar among these experiments; nevertheless, milk production responses to the supplement varied from a decrease of 1.7 kg/d to an increase of 3.6 kg/d (Table 2). Looking at the plasma concentrations of both vitamins as indicators of the vitamin status of the animals, it appears that the largest response was observed in experiment 3 where plasma concentrations of both vitamins were the lowest whereas the negative response was observed in experiment 5 where both concentrations were the highest. These observations suggest that at least part of the variability among experiments studying production and metabolic responses to B-vitamin supplements could be due to the vitamin status of the cow which reflects vitamin supply. Indeed, when the vitamin supply is adequate, a supplementation is likely to be useless.

The Challenge: Estimating B-vitamin Supply

Table 3 illustrates the great variability of intake, duodenal flow and apparent synthesis of B vitamins in the rumen of dairy cows. Negative values for apparent ruminal synthesis indicate that the amount of vitamin destroyed in the rumen is greater than the amount of vitamin ingested. As B-vitamin absorption takes place mostly in the small intestine, the duodenal flow of B vitamins represents the amount of vitamins available for absorption by the cow.

In non-ruminants, B-vitamin supply can be calculated by multiplying B-vitamin concentrations in the diet by the intake. In ruminants, B-vitamin supply is the sum of the vitamins ingested and not destroyed by the ruminal microbial population and those synthesized in the rumen.

In the experiments reported in Figure 1, 6 diets based on alfalfa silage (range of 42 to 60% on a DM basis), dry corn (range of 34 to 39% on a DM basis) and soybean meal and/or SoyPlus (range of 4 to 13% on a DM basis) were fed to lactating dairy cows (Castagnino et al., 2016a, b, c). Folate intake was similar for diets C and D, 11 mg/d, but the amount of folates recovered at the duodenum was 75% with diet C compared with the amount recovered with diet D (**Figure 1a**). Moreover, as shown in Figure 1, all B vitamins did not respond alike to dietary changes. Among the studied diets, niacin intake was nearly ten times greater for diet C than A but the amount of niacin reaching the small intestine was similar, 1197 vs. 1268 mg/d for diets A and C, respectively (**Figure 1b**). In the present example, apparent ruminal synthesis of niacin seems to be inversely proportional to the amount ingested.

Figure 1 illustrates why, in dairy cows, the amount of B vitamins ingested is not a reliable indicator of the amount of vitamins reaching the sites of absorption and available for the animal. It also highlights the fact that effects on one vitamin cannot be extrapolated to another one. Knowledge on the factors controlling the amounts of B vitamins escaping the rumen is very limited. It is likely that ingredient and diet composition and their consequences on ruminal fermentation pattern control the fate of B vitamins in the rumen. Increasing knowledge on these effects possibly offers the best approach to predict B vitamin supply for the dairy cow.

Conclusions

Research on B-vitamin requirements of dairy cows is still in its very early stage. The number of published experiments on production and metabolic responses of dairy cattle to B-vitamin supplements is still very small. As described above, there is scientific evidence that B-vitamin supply from the diet and synthesis in the rumen is not always sufficient to meet the needs because increasing supply in niacin, biotin, folic acid and vitamin B_{12} improves metabolic efficiency of dairy cows, especially during the critical period around calving and in early lactation. One has to remember however, that these values are the doses most frequently used in the published experiments; they are not requirements, they cannot even be considered as recommended intakes because even

for the most studied vitamins, very few dose-response experiments have been conducted. Moreover, although quantification of the metabolic demand for B vitamins is still far from precise, variability among experiments is likely frequently due to differences in the amounts of B vitamins available for the cow. Consequently, recommendations for B-vitamin adequate intakes is dependent of our ability to predict their total supply, i.e. the amounts of vitamins from dietary sources escaping degradation in the rumen plus the amounts synthesized in the rumen. If supply and requirement are equal, a positive effect of a B-vitamin supplement is unlikely whereas a positive response to supplementation can be expected if the supply is sub-optimal.

References

Akins, M. S., S. J. Bertics, M. T. Socha, and R. D. Shaver. 2013. Effects of cobalt supplementation and vitamin B_{12} injections on lactation performance and metabolism of Holstein dairy cows. J. Dairy Sci. 96:1755-1768.

Aschemann, M., P. Lebzien, L. Hünter, K.-H. Südekum, and S. Dänicke. 2012. Effect of niacin supplementation on rumen fermentation characteristics and nutrient flow at the duodenum in lactating dairy cows fed a diet with a negative rumen nitrogen balance. Arch. Anim. Nutr. 66:303-318.

Beaudet, V., R. Gervais, B. Graulet, P. Nozière, M. Doreau, A. Fanchone, D. S. Castagnino and C. L. Girard. 2016. Effects of dietary nitrogen levels and carbohydrate sources on apparent ruminal synthesis of some B vitamins in dairy cows. J. Dairy Sci. 99:2730-2739.

Bechdel, S. I., H. E. Honeywell, R. A. Dutcher, and M. H. Knutsen. 1928. Synthesis of vitamin B in the rumen of the cow. J. Biol. Chem. 80:231-238.

Bills, N. D., M. J. Koury, A. J. Clifford, and E. N. Dessypris. 1992. Ineffective hematopoiesis in folate-deficient mice. Blood. 79:2273-2280.

Brent, B. E., and E. E. Bartley. 1984. Thiamin and niacin in the rumen. J. Anim. Sci. 59:813-822.

Breves, G., M. Brandt, H. Hoeller, and K. Rohr. 1981. Flow of thiamin to the duodenum in dairy cows fed different rations. J. Agric. Sci., Camb. 96: 587-591.

Castagnino, D. S., M. Seck, V. Beaudet, K. L. Kammes, J. A. Voelker Linton, M. S. Allen, R. Gervais, P. Y. Chouinard, and C. L. Girard. 2016a. Effects of forage family on apparent ruminal synthesis of B vitamins in lactating dairy cows. J. Dairy Sci. 99:1884-1894.

Castagnino, D. S., K. L. Kammes, M. S. Allen, P. Y. Chouinard, and C. L. Girard. 2016b. Particle length of silages affects apparent ruminal synthesis of B vitamins in lactating dairy cows. J. Dairy Sci. 99: 6229-6236.

Castagnino, D. S., K. L. Kammes, M. S. Allen, R. Gervais, P. Y. Chouinard, and C. L. Girard. 2016c. High-concentrate diets based on forages harvested at different maturity stages affect ruminal synthesis of B vitmains in lactating dairy cows. Animal. Published online: 30 August 2016, pp. 1-8. DOI: http://dx.doi.org/10.1017/S1751731116001798

Chen, B., C. Wang, Y. M. Wang, and J. X. Liu. 2011. Effect of biotin on milk performance of dairy cattle: A meta-analysis. J. Dairy Sci. 94:3537-3546.

Combs, G. F. 2012. The Vitamins. Fundamental aspects in nutrition and health. 4th edition. Academic Press, Elsevier Inc., USA.
Croom, W. J., A. H. Rakes, A. C. Linnerud, G. A. Ducharme, and J. M. Elliot. 1981. Vitamin B_{12} administration for milk fat synthesis in lactating dairy cows fed a low fiber diet. J. Dairy Sci. 64:1555-1560.
Dennis, S. M., M. J. Arambel, E. E. Bartley, D. O. Riddell, and A. D. Dayton. 1982. Effect of heated or unheated soybean meal with or without niacin on rumen protozoa. J. Dairy Sci. 65:1643-1646.
Di Costanzo, A., J. N. Spain, and D. E. Spiers, 1997. Supplementation of nicotinic acid for lactating Holstein cows under heat stress conditions. J. Dairy Sci. 80:1200–1206.
Duplessis, M., C. L. Girard, D. E. Santschi, D. M. Lefebvre, and D. Pellerin. 2014a. Milk production and composition, and body measurements of dairy cows receiving intramuscular injections of folic acid and vitamin B_{12} in commercial dairy herds. Livest. Sci. 167: 186-194.
Duplessis, M., C. L. Girard, D. E. Santschi, J.-P. Laforest, J. Durocher, and D. Pellerin. 2014b. Effects of folic acid and vitamin B_{12} supplementation on culling rate, diseases, and reproduction in commercial dairy herds. J. Dairy Sci. 97:2013-7369.
Duplessis, M., D. Pellerin, R. I. Cue, and C. L. Girard. 2016. Short communication: Factors affecting vitamin B12 concentration in milk of commercial dairy herds: An exploratory study. J. Dairy Sci. 99: 4886-4892.
Elliot, J. M., D. E. Hogue, H. F. Tyrrell. 1965. Blood vitamin B_{12} status of the dairy cow in late pregnancy and early lactation. J. Dairy Sci. 48:1335-1338.
Erickson, P. S., A. M. Trusk, and M. R. Murphy. 1990. Effects of niacin source on epinephrine stimulation of plasma nonesterified fatty acid and glucose concentrations, on diet digestibility and on rumen protozoal numbers in lactating dairy cows. J. Nutr. 120:1648-1653.
Ferreira, G., W. P. Weiss, and L. B. Willett. 2007. Changes in Measures of Biotin Status Do Not Reflect Milk Yield Responses When Dairy Cows Are Fed Supplemental Biotin. J. Dairy Sci. 90:1452-1459.
Frobish, R. A., and C. L. Davis. 1977. Theory involving propionate and vitamin B_{12} in the low-milk fat syndrome. J. Dairy Sci. 60:268-273.
Fukuwatari, T., and K. Shibata. 2013. Nutritional aspects of tryptophan metabolism. Internat. J. Tryp. Res. 6 (suppl. 1): 3-8.
Gagnon, A., D. R. Khan, M. A. Sirard, C. L. Girard, J.-P. Laforest, and F. J. Richard. 2015. Effects of intramuscular administration of folic acid and vitamin B_{12} on granulosa cells gene expression in postpartum dairy cows. J. Dairy Sci. 98:7797-7809.
Ghaemialehashemi, S. 2013. Effet d'injections hebdomadaires d'un combiné d'acide folique et de vitamine B_{12} sur la reprise de l'activité ovarienne postpartum chez les vaches laitières. Mémoire de Maîtrise. Université Laval, Québec, QC.
Girard, C. L., and J. J. Matte. 1998. Dietary supplements of folic acid during lactation; effects on the performance of dairy cows. J. Dairy Sci. 81:1412-1419.
Girard, C. L., and J. J. Matte. 1999. Changes in serum concentrations of folates, pyridoxal, pyridoxal-5-phosphate and vitamin B_{12} during lactation of dairy cows fed dietary supplements of folic acid. Can. J. Anim. Sci. 79:107-113.

Girard, C. L., and J. J. Matte. 2005. Effects of intramuscular injections of vitamin B_{12} on lactation performance of dairy cows fed dietary supplements of folic acid and rumen-protected methionine. J. Dairy Sci. 88:671-676.
Girard, C. L., H. Lapierre, J. J. Matte, and G. E. Lobley. 2005. Effects of dietary supplements of folic acid and rumen-protected methionine on lactational performance and folate metabolism of dairy cows. J. Dairy Sci. 88:660-670.
Girard, C. L., C. Benchaar, J. Chiquette, and A. Desrochers. 2009. Net flux of nutrients across the rumen wall of lactating dairy cows as influenced by dietary supplements of folic acid. J. Dairy Sci. 92:6116-6122.
Graulet, B., J. J. Matte, A. Desrochers, L. Doepel, M. F. Palin, and C. L. Girard. 2007. Effects of dietary supplements of folic acid and vitamin B_{12} on metabolism of dairy cows in early lactation. J. Dairy Sci. 90:3442-3455.
Hoppner, J. H., and B. C. Johnson. 1955. The production and study of an acute nicotinic acid deficiency in the calf. J. Nutr. 56:303-310.
Horner, J. L., C. E. Coppock, J. R. Moya, J. M. Labore, and J. K. Lanham. 1988a. Effects of niacin and whole cottonseed on ruminal fermentation, protein degradability, nutrient digestibility. J. Dairy Sci. 71:1239-1247.
Horner, J. L., L. M. Windle, C. E. Coppock, J. M. Labore, J. K. Lanham, and D. H. Nave. 1988b. Effects of whole cottonseed, niacin, and niacinamide on in vitro rumen fermentation and on lactating Holstein cows. J. Dairy Sci. 71:3334-3344.
Kincaid, R. L., and M. T. Socha. 2007. Effect of cobalt supplementation during late gestation and early lactation on milk and serum measures. J. Dairy Sci. 90:1880-1886.
Lean, I. J., and A. R. Rabiee. 2011. Effect of feeding biotin on milk production and hoof health in lactating dairy cows: A quantitative assessment. J. Dairy Sci. 94:1465-1476.
Lebzien, P., H. Abel, B. Schröder, and G. Flachowsky. 2006. Studies on the biotin flow at the duodenum of dairy cows fed diets with different concentrate levels and type of forages. Arch. Anim. Nutr. 60:80-88.
Li, H. Q., Q. Liu, C. Wang, Z. M. Yang, G. Guo, W. J. Huo, C. X. Pei, Y. L. Zhang, S. L. Zhang, H. Wang, J. X. Liu, and Y. X. Huang. 2016. Effects of dietary supplements of rumen-protected folic acid on lactation performance, energy balance, blood parameters and reproductive performance in dairy cows. Anim. Feed Sci. Technol. 213:55-63.
Lohölter, M., U. Meyer, C. Rauls, J. Rehage, and S. Dänicke. 2013. Effects of niacin supplementation and dietary concentrate proportion on body temperature, ruminal pH and milk performance of primiparous dairy cows. Arch. Anim. Nutr. 67:202-218.
Martens, J. H., H. Barg, M. Warren, and D. Jahn. 2002. Microbial production of vitamin B_{12}. Appl. Microbiol. Biotechnol. 58:275-285.
McDowell, L. R. 2000. Vitamins in animal and human nutrition. 2nd ed. Iowa State University Press, Ames. IA.
Menzies, K. K., C. Lefèvre, J. A. Sharp, K. L. Macmillan, P. A. Sheehy, and K. R. Nicholas. 2009. A novel approach identified the FOLR1 gene, a putative regulator of milk protein synthesis. Mamm Genome. 20:498–503.
Mykkänen, H. M., and H. Korpela. 1981. Serum vitamin B_{12} levels in dairy cows before and after parturition. Zbl. Vet. Med. A. 28:526-528.

Niehoff, I.-D., L. Hüther, P. Lebzien, W. Bigalke, S. Dänicke, and G. Flachowsky. 2009. Investigations on the effect of a niacin supplementation to three diets differing in forage-to-concentrate ration on several blood and milk variables of dairy cows. Arch. Anim. Nutr. 63:203-218.

Niehoff, I.-D., L. Hüther, P. Lebzien, and G. Flachowsky. 2013. The effect of a niacin supplementation to different diets on ruminal fermentation and flow of nutrients to the duodenum of dairy cows. Landbauforsch – Appl. Agric. Forestry Res. 63:143-154.

Ottou, J. F., and M. Doreau. 1996. Influence of niacin on in vitro ruminal fermentation and microbial synthesis depending on dietary factors. Anim. Feed Sci. Technol. 58:187-199.

Pineda, A., J. K.Drackley, J. Garrett, and F. C. Cardoso. 2016. Effects of rumen-protected niacin on milk production and body temperature of middle and late lactation Holstein cows. Livest. Sci. 187:16–23.

Pires, J. A. A., L. F. Stumpf, I. D. Soutullo, J. B. Pescara, S. E. Stocks, and R. R. Grummer. 2016. Effects of abomasal infusion of nicotinic acid on responses to glucose and B-agonist challenge in underfed lactating cows. J. Dairy Sci. 99:2297–2307

Preynat, A., H. Lapierre, M. C. Thivierge, M. F. Palin, J. J. Matte, A. Desrochers, and C. L. Girard. 2009a. Influence of methionine supply on the responses of lactational performance of dairy cows to supplementary folic acid and vitamin B_{12}. J. Dairy Sci. 92:1685-1695.

Preynat, A., H. Lapierre, M. C. Thivierge, M.-F. Palin, J. J. Matte, A. Desrochers, and C. L. Girard. 2009b. Effects of supplements of folic acid, vitamin B_{12}, and rumen-protected methionine on whole body metabolism of methionine and glucose in lactating dairy cows. J. Dairy Sci. 92:677-689.

Riddell, D. O., E. E. Bartley, and A. D. Dayton. 1980. Effect of nicotinic acid on rumen fermentation in vitro and in vivo. J. Dairy Sci. 63:1429-1436.

Riddell, D. O., E. E. Bartley, and A. D. Dayton. 1981. Effect of nicotinic acid on microbial protein synthesis in vitro and on dairy cattle growth and milk production. J. Dairy Sci. 64:782-791.

Rosendo, O., C. R. Staples, L. R. McDowell, R. McMahon, L. Badinga, F. G. Martin, J. F. Shearer, W. M. Seymour, N. S. Wilkinson. 2004. Effects of biotin supplementation on peripartum performance and metabolites of Holstein cows. J. Dairy Sci. 87: 2535-2545.

Rungruang, S., J. L. Collier, R. P. Rhoads, L. H. Baumgard, M. J. de Veth, and R. J. Collier. 2014. A dose-response evaluation of rumen-protected niacin in thermoneutral or heat-stressed lactating Holstein cows. J. Dairy Sci. 97:5023-5034.

Santschi, D. E., R. Berthiaume, J. J. Matte, A. F. Mustafa, and C. L. Girard. 2005. Fate of supplementary B-vitamins in the gastrointestinal tract of dairy cows. J. Dairy Sci. 88:2043-2054.

Schüssler, S. L., G. C. Fahey jr., J. B. Robinson, S. S. Masters, S. C. Loerch, and J. W. Spears. 1978. The effect of supplemental niacin on in vitro cellulose digestion and protein synthesis. Int. J. Vit. Nutr. Res. 48:359-367.

Schwab, E. C., D. Z. Caraviello, and R. D. Shaver. 2005. Review: A meta-analysis of lactation responses to supplemental dietary niacin in dairy cows. Prof. Anim. Sci. 21:239-247.

Schwab, E. C., C. G. Schwab, R. D. Shaver, C. L. Girard, D. E. Putnam, and N. L. Whitehouse. 2006. Dietary forage and nonfiber carbohydrate contents influence B-vitamin intake, duodenal flow, and apparent ruminal synthesis in lactating cows. J. Dairy Sci. 89:174-187.

Scott, J.M. 1999. Folate and vitamin B_{12}. Proceedings of the Nutrition Society 58:441–448.

Seck, M., J. A. Voelker Linton, M. S. Allen, D. S. Castagnino, P. Y. Chouinard, and C. L. Girard. 2016. Apparent ruminal synthesis of B vitamins in lactating dairy cows fed diets with different forage-to-concentrate ratios. J. Dairy Sci. Accepted, November 21, 2016.

Shastri, N. V., S. G. Nayudu, and M. C. Nath. 1968. Effects of high fat and high fat-high protein diets on biosynthesis of niacin from tryptophan in rats. J. Vitaminol. 14:198-202.

Shields, D. R., D. M. Schaefer, and T. W. Perry. 1983. Influence of niacin supplementation and nitrogen source on rumen microbial fermentation. J. Anim. Sci. 57:1576-1583.

Steinberg, von W., and W. Kaufmann. 1977. Untersuchungen zur bakteriellen Thiaminsynthese in den Vormägen von Milchkühen [Investigations on the bacterial thiamin synthesis in the rumen of dairy cows]. Z. Tierphysiol., Tierernährg. U Futtermittenlkde. 39:298-301.

Waterman, R., L. H. Schultz. 1972. Nicotinic acid loading of normal cows: effects on blood metabolites and excretory forms. J. Dairy Sci. 55:1511-1513.

Waterman, R., J. W. Schwalm, and L. H. Schultz. 1972. Nicotinic acid treatment of bovine ketosis. I. Effects on circulatory metabolites and interrelationships. J. Dairy Sci. 55:1447-1453.

Wrinkle, S. R., P. H. Robinson, and J. E. Garrett. 2012. Niacin delivery to the intestinal absorptive site impacts heat stress and productivity responses of high producing dairy cows during hot conditions. Anim. Feed Sci. Technol. 175:33-47.

Yuan, K., R. D. Shaver, S. J. Bertics, M. Espineira, and R. R. Grummer. 2012. Effect of rumen-protected niacin on lipid metabolism, oxidative stress, and performance of transition dairy cows. J. Dairy Sci. 95:2673–2679.

Zimbelman, R. B., L. H. Baumgard, and R. J. Collier. 2010. Effects of encapsulated niacin on evaporative heat loss and body temperature in moderately heat-stressed lactating Holstein cows. J. Dairy Sci. 93: 2387-2394.

Zimbelman, R. B., R. J. Collier, and T. R. Bilby. 2013. Effects of utilizing rumen protected niacin on core body temperature as well as milk production and composition in lactating dairy cows during heat stress. Anim. Feed Sci. Technol. 180:26-33.

Table 1. Effects of biotin supplements on lactation performance of dairy cows – summary of two meta-analyses.

	Lean and Rabie (2011)		Chen et al. (2011)	
	Difference	*P* value	Difference	*P* value
Dry matter intake, kg/d	+0.70	0.09	+0.87	0.01
Milk production, kg/d	+1.29	0.002	+1.66	0.002
Milk fat, %	+0.05	0.23	+0.01	0.53
Milk protein, %	-0.09	0.33	+0.03	0.55
Milk fat, kg/d	0.07	0.08	+0.05	0.04
Milk protein, kg/d	0.02	0.09	+0.05	0.001

Table 2. Milk production responses to the administration of a combined supplement of folic acid and vitamin B_{12} in early lactation in 5 experiments.

	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
Control					
Dry matter intake, kg/d	21.3	21.8	21.1	22.5	21.0
Milk production, kg/d	39.5	37.7	40.1	39.5	41.5
Effect of the combined supplement of vitamins on milk production, kg/d					
	+1.4	+1.2	+3.6	+1.2	-1.7
Control					
Plasma folates, ng/mL	15	16	11	11	16
Plasma vitamin B_{12}, pg/mL	172	181	131	223	216

Table 3. Intake, duodenal flow and apparent ruminal synthesis of B vitamins (mg/kg of dry matter intake) [1].

	Intake	Apparent synthesis in the rumen	Duodenal flow
Thiamin	1.3 to 3.8	-1.5 to 4.2	0.8 to 7.8
Riboflavin	4 to 106	-50 to 29	3 to 87
Niacin	22 to 170	-123 to 120	47 to 146
Pantothenic acid [2]			
Vitamin B_6	2.6 to 17.6	-14.1 to 1.3	0.7 to 7.7
Biotin	0.2 to 7.0	-0.9 to 0.2	0.2 to 6.6
Folates	0.2 to 1.1	0.5 to 3.3	0.9 to 2.4
Vitamin B_{12} [3]	-[4]	0.1 to 4.8	0.1 to 4.8

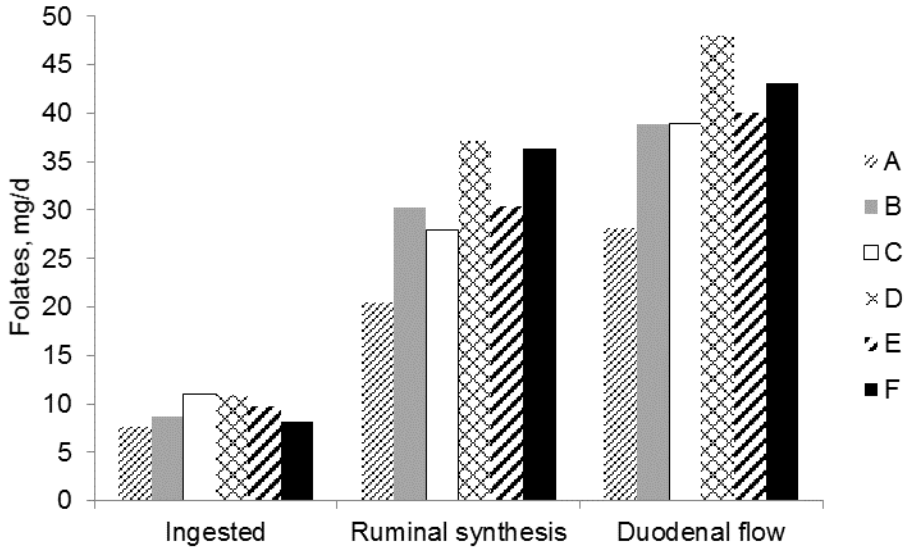
[1] Breves et al., 1981; Steinberg and Kaufman, 1977; Santschi et al., 2005; Schwab et al., 2006; Lebzien et al., 2006; Niehoff et al., 2013; Beaudet et al., 2016; Castagnino et al., 2016a, b, c; Seck et al., 2016.

[2] No data available.

[3] Total dietary concentrations of cobalt: 0.17 to 2.5 mg/kg DM.

[4] Under or close to the level of detection.

a)



b)

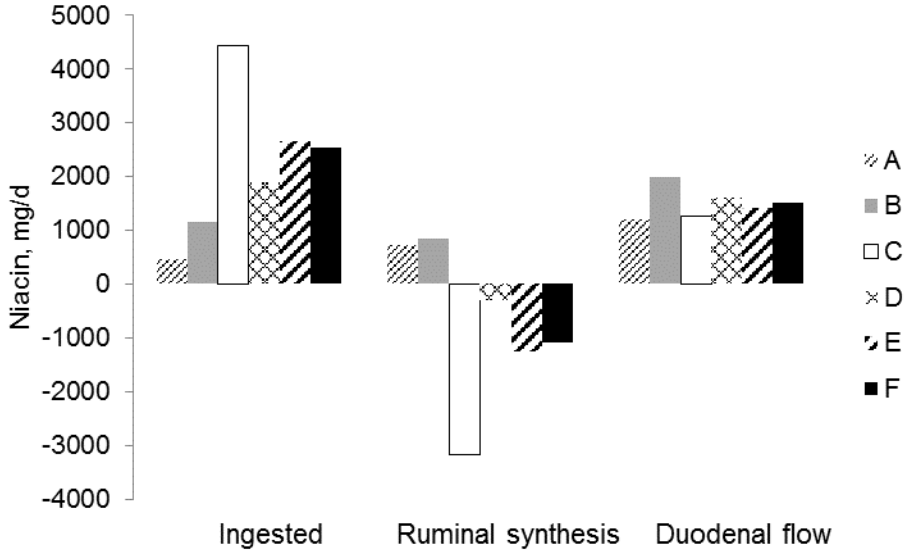


Figure 1. Daily intake, apparent ruminal synthesis and duodenal flow of a) folates and b) niacin in dairy cows fed 6 diets based on alfalfa silage.

DCAD: It's Not Just for Dry Cows

Rich Erdman[1] and Marie Iwaniuk
Department of Animal and Avian Sciences, University of Maryland

Introduction

For more than 20 years, dairy producers have been using low DCAD diets in their dry cow feeding programs to prevent milk fever and subclinical hypocalcemia during the transition period. The use of low DCAD diets in dry cows has virtually eliminated the incidence of milk fever in most dairy herds. While dairy producers are well aware of the importance of proper DCAD concentrations in the dry period, relatively little attention has been paid to the effect of DCAD in lactating cows. We will review the principles of the strong ions in physiology and calculating and formulating for DCAD and then highlight the responses of lactating cows to DCAD.

What is DCAD?

The term **DCAD** stands for Dietary Cation Anion Difference. DCAD is an index of the relative balance between the principle cations (potassium, K and sodium, Na) and the principle anions (chloride, Cl and sometimes sulfur, S) in the cow's diet. Sodium, potassium, and chloride fall into a class of dietary minerals that are sometimes referred to as the "osmoregulators" because of the critical role that they play in maintaining osmotic balance in various body tissues (**Table 1**). In blood, Na is the primary cation and Cl (and to a lesser extent, bicarbonate ion) are the primary anions. In the cell, K is the principal cation while amino acids and proteins with a negative charge serve as the principle anions. Finally, in ruminal fluid, a combination of Na and K are the principal cations whereas volatile fatty acids (**VFA**) that are produced during ruminal fermentation serve as the primary anions. These minerals are absorbed from the diet with nearly 100% efficiency and can readily move across the intestinal wall, blood, and cell membranes. Their relative content in these tissues is maintain by a Na-K-ATP pump. They are also important for maintaining osmotic balance in milk and the relatively consistent moisture content (85%) of feces in the cow. Finally, any excess of these ions is excreted in the urine. Sodium and potassium are the primary drivers of urine output and thus added intake will also increase water intake in the cow.

There are two important principles with respect to the cations and anions: 1) the sum of the cations and anions (equivalent weight basis) should add up to about 300 to maintain a consistent osmotic pressure and maintain water balance between tissues; and 2) the sum of the cations should equal the sum of the anions to maintain neutral electrical charge. These two principles are important in understanding the role of DCAD in acid-base balance and urinary excretion of these minerals.

[1] Contact: Department of Animal and Avian Sciences, University of Maryland, Bldg. 142. College Park, MD 20742. (301) 405-4243. Email: erdman@umd.edu

Table 1. Principle cations and anions (mEq/L) in bodily fluids.

Ion(charge)	Blood	Intracellular	Ruminal fluid
Sodium (Na^+)	145	12	84
Potassium (K^+)	4	139	27
Chloride (Cl^-)	116	4	8
Bicarbonate (HCO_3^-)	29	12	6
Amino acids & proteins$^-$	9	138	(VFA's) 105
Magnesium (Mg++)	1.5	0.8	4.2[1]
Calcium (Ca++)	1.8	< 0.0002	3.5[1]
Milliosmoles/L	290	290	315[1]

[1] From Bennink et al. (1978).

The Strong Ion Theory

Sodium, potassium, and chloride are also referred to as the "Strong Ions" because they are absorbed from the diet with nearly 100% efficiency, they remain completely dissociated in solution, and physiologically, any surplus intake from the diet above an animal's needs will be excreted in the urine. The "Strong Ion Theory of Acid-Base Balance," first proposed by Peter Stewart, a Canadian physiologist (Stewart, 1978) applies to virtually every mammal including humans. Stewart (1978) referred to the sum of the strong cations minus the sum of the anions as the Strong Ion Difference (**SID**):

$$SID = Na^+ + K^+ - Cl^-$$

The SID equation is in fact identical to the simplest DCAD equation that was first developed for poultry and swine that is also referred to as the Mongin (1981) equation. Excretion of strong ion secretion in the urine can be summarized by the following equation where the sum of the cations (Na^+, K^+, H^+) must equal the sum of the anions (Cl^-, OH^-) to maintain electrochemical neutrality:

$$Na^+ + K^+ + H^+ = Cl^- + OH^-$$

If an animal consumes a diet that is high in cations in relation to anions (SID or DCAD is positive), its urine must contain additional anions to maintain electrochemical neutrality. Cattle routinely consume diets that are high in K. The additional base (anion) excreted in the urine is usually the bicarbonate ion. In contrast, cattle consuming diets that are high in Cl relative to K and Na (DCAD or SID is negative), additional cations such as ammonium (NH_4^+) and other titratable acids are needed to balance the negative charge of Cl. Because of this relationship, animals such as cattle which are typically fed diets high in cations will have an alkaline urine (pH > 7) whereas animals that are fed diets that are low in cations will have acid urine (pH < 7). This concept is

illustrated in **Table 2** that compares lactating sows and dairy cows. Pigs, because they consume a low K diet, have an acidic urine whereas cows that consume a high K diet have an alkaline urine.

Table 2. Comparison of strong ion requirements for lactating dairy cows and sows using the 2001 Dairy NRC and 2012 Swine NRC.

Mineral	Lactating sow requirement % of diet, as-fed	Lactating cow requirement % of dietary DM
Na	0.20	0.23
K	0.20	1.06
Cl	0.16	0.24
DCAD, mEq/kg	93	303
Expected urine pH	6.5	7.5 to 8.0

How Does DCAD Work in Preventing Milk Fever?

The initial work on use of DCAD was based on the observation by Scandinavian researchers that cows fed diets that were low in ash content resulted in reduced incidence of milk fever (Ender et al.,1971; Dishington et al., 1975). Since K is a major factor that affects ash content (low ash diets were also low in K), it was found that diets with low DCAD (low K and Na relative to Cl) reduced not only milk fever but also subclinical hypocalcemia. Since excess dietary Cl is excreted in urine, it requires a corresponding cation to maintain a neutral charge. Low K diets stimulated hydrogen ion (low pH) secretion and the "spilling of calcium" (Ca^{++}) in the urine. In turn, increased loss of Ca in the urine also increased the cow's metabolic mechanisms for increased resorption of Ca from bone and increased intestinal absorption of Ca from the diet such that the cow was able to regulate blood Ca more effectively when the increased demand for Ca in milk production kicked in at the time of calving.

These observations stimulated numerous studies on the use of DCAD to prevent milk fever by Elliott Block at McGill University in Canada, Jesse Goff and Ron Horst at the USDA Animal Disease Laboratory in Iowa, and several others. The key points from their work were the following: 1) Diets that were negative in DCAD were effective in preventing milk fever and subclinical hypocalcemia; 2) Selection of feeds that were low in K and Na along with addition of Cl and sulfate salts were required to achieve low or negative DCAD diets; and 3) Low urine pH was a very useful indicator of the cow's DCAD status. Probably the most pivotal experiment was a study using Jersey cows by Goff and Horst (1997) in which cows were fed diets containing 1.1, 2.1, or 3.1% K with either 0.5 or 1.5% Ca during the dry period. The DCAD across Ca levels was increased from -75 to +430 mEq/kg of dietary DM with increasing K. Incidence of milk fever increased from 0% in the 1.1% K, 0.5% Ca diet to 80% in the 3.1% K with either 0.5 or 1.5% Ca. It was clear that the low DCAD (low K) diets had a profound effect on milk

fever. Subsequent work looked at the effectiveness of various Cl and sulfate salts to reduce urine pH and it was determined that dietary sulfur was about 60% as effective as Cl in reducing urine pH and preventing hypocalcemia (Goff et al., 2004).

The DCAD Equations

The simplest calculation of DCAD is referred to as the Mongin (1981) equation that was originally developed for formulating poultry and swine diets. The formula includes the Na, K, and Cl content of the diet. An example of DCAD calculations for a diet that meets the minimum (NRC, 2001) requirements for K, Na, and Cl in lactating dairy cows is illustrated in **Table 3**. The DCAD is most frequently expressed as either mEq/kg of mEq/100 g of feed DM. The difference in magnitude is a factor of 10.

Table 3. Calculation of DCAD for a lactating dairy cow diet containing the minimum concentrations of K, Na, and Cl (NRC, 2001).

Element	% of DM	g/kg	Atomic Wt., g	Eq./kg	mEq/kg
K	1.06	12	39.1	0.271	271
Na	0.23	2.3	23.0	0.100	100
Cl	0.24	2.5	35.5	0.067	67

DCAD = mEq K + mEq Na – mEq Cl

DCAD = 271 + 100 – 67

DCAD = 304 mEq per kg of DM

= 30.4 mEq per 100 g of DM

Table 4 shows the various DCAD equations that have been used by dairy nutritionists in diet formulation programs. Each equation is very similar in that they all account for the strong ion (K, Na, and Cl) content of the diet. The first equation suggested for use in formulating dry cow diets was proposed by Ender (1971). This equation includes dietary sulfur (S) which has a +2 valence; therefore, in this equation, the S content divided by the atomic weight is multiplied by 2. The inclusion of S in the DCAD formula is only important when dietary S varies. Typically, this is not an issue unless distillers grains (**DDGS**) are a major component of the cow's diet. As stated earlier, the Mongin equation is the simplest equation and is equally effective as long as dietary S does not vary substantially. The NRC (2001) equation is perhaps the most precise and is based on the relative absorption rate of each of the minerals in the equation. However, very few nutritionists utilize that equation. Finally the Goff et al (2004) equation with a 0.6 coefficient for S is based on the relative effectiveness of sulfate salts in reducing urine pH compared to Cl salts. In my opinion, this is probably the most precise of all of the DCAD equations. However, the Ender (1971) DCAD

equation still remains the most commonly used one in spite of the fact that it probably overemphasizes the role of dietary S.

Table 4. Examples of various DCAD equations used in dairy cattle feeding programs.

Equation	Elements Included:	DCAD, mEq/kg of DM
Ender (1971)	Na + K - Cl - S	179
Mongin (1981)	Na + K - Cl	304
2001 Dairy NRC	(Na + K + 0.15 Ca + 0.15 Mg) − (Cl + 0.6 S + 0.5 P)	284
Goff et al. (2004)	Na + K - Cl - 0.6S	228

DCAD in Lactating Dairy Cow Diets

Although negative DCAD diets have been fed to dry cows for many years, relatively little work was done on the effect of DCAD in lactating dairy cow diets until the late 1980's and early 1990's. Work by Tucker et al. (1988) demonstrated that, in contrast to dry cows, negative DCAD diets should not be fed to lactating cows because negative DCAD diets resulted in reduced feed intake and milk production. A series of experiments at Georgia (West et al., 1992) and Florida (Sanchez and Beede, 1996) examined the effects DCAD during heat stress. They suggested that increasing DCAD improved feed intake, milk production, and milk fat concentration during heat stress. The importance of DCAD was extensively discussed in the 2001 NRC publication but no minimal DCAD requirement was established. There simply had not been enough experiments conducted with varying DCAD concentrations to establish a requirement at the time publication. If one were to feed diets at the minimal requirements for K, Na, Cl, and S, the implied requirement would be around 179 mEq/kg of DM using the Ender (1971) equation that includes dietary S or about 304 mEq/kg of DM using the Mongin (1981) equation that does not include S in the formula.

The first meta-analysis of DCAD studies in lactating dairy cows was published by Hu and Murphy (2004) in which the results of 12 papers involving 17 experiments and 54 treatment means were summarized. Hu and Murphy (2004) estimated that maximum feed intake, milk production, and 4% fat-corrected milk (**FCM**) production occurred at DCAD's of 40, 34, and 49 mEq/100 g of feed DM, respectively using the Mongin (1981) equation to calculate DCAD. This study conclusively demonstrated the importance of feeding positive DCAD diets to lactating cows. However, the number of experiments and treatment means available for the analysis were limited. Further, many of the diets in that summary were DCAD negative with more than 50% of the treatment means from cows fed diets containing less than 304 mEq/kg of DM, the theoretical requirement for cows fed diets with the minimum requirements for K, Na, and Cl. Because Hu and Murphy (2004) had chosen to use a quadratic equation to explain the data, only a maximal response to DCAD rather than an optimal response could be determined.

Dietary buffers containing bicarbonate and carbonate salts of K and Na will increase DCAD and they have been a common feed additive in dairy cow diets for more than 50 years. We reasoned that the numerous feeding studies on the use of buffers in the transition period and to increase milk fat in low forage diets (Erdman, 1988) along with studies published since 2004 could be used to augment the dataset of Hu and Murphy (2004). Although some of the older publications did not have complete mineral analysis to calculate DCAD, we were able to show that book values from the 2001 NRC software could be used to fill in the missing mineral concentrations and accurately predict DCAD (Iwaniuk and Erdman, 2015). The calculated DCAD from those publications were the basis for our recent meta-analysis of DCAD effects on lactating dairy cows (Iwaniuk and Erdman, 2015). A total of 43 articles published between 1965 and 2011 that included 196 treatment means and 89 DCAD treatment comparisons were included in the analysis. The range in DCAD was from −68 to +811 mEq/kg of dietary DM (Ender equation), but the vast majority of diets contained between 0 and 500 mEq/kg of dietary DM, which we considered to be the practical range of inference. Figure 1 (A to D) shows a summary of the dry matter intake (**DMI**), milk production, and milk composition responses to DCAD from that analysis that were fitted to curvilinear and linear response equations. For DMI (**Figure 1A**), the maximum response was 1.92 kg/d (4.2 lb/d); 66% and 80% of the maximum DMI responses were achieved at DCAD concentrations of 290 and 425 concentrations, respectively. Maximum milk production responses (**Figure 1B**) were small (1.1 kg/d; 2.4 lb/d) with very little response to DCAD above 300 mEq/kg of dietary DM. For milk fat percentage and yield (**Figures 1C and 1D**, respectively), the responses were linear. Every 100 mEq/kg increase in DCAD resulted in a 1 point (0.1 percentage unit) increase in milk fat percent and a 38 g/d (0.08 lb/d) increase in milk fat yield. This suggests that fat yield will be the primary economic response to DCAD. Consequently, the 3.5% FCM response was much greater than for milk production alone and that the 66% and 80% of the maximum FCM response (4.8 kg/d, 10.8 lb/d) occurred at DCAD concentrations of 450 and 675 mEq/kg of DM, respectively. We consider the 675 mEq/kg of dietary DM DCAD value to be outside of the range of inference of this data set. There were no effects of DCAD on milk protein percent or yield (data not shown). In summary, clearly there are intake, milk production, and milk composition responses to DCAD and these effects need to be accounted for in diet formulation for lactating dairy cows.

We also looked at the effects of DCAD on ruminal fluid pH (data not shown). A 100 mEq/kg of dietary DM increase in DCAD resulted in a linear 0.003 unit increase in ruminal fluid pH such that increasing DCAD from 0 to 500 mEq/kg of dietary DM was projected to increase mean ruminal fluid pH from 6.31 to 6.46. These results are very consistent with earlier studies on the use of buffers to increase ruminal fluid pH and correspond to changes in milk fat percent (Iwaniuk and Erdman, 2015).

With respect to digestibility, increasing DCAD from 0 to 500 mEq/kg of dietary DM resulted in a 3.5 percentage unit increase in DM digestibility and a 7.5 percentage unit increase in NDF digestibility (**Figures 2A & B**). About two thirds of the increase in DM digestibility was due to increased NDF digestibility. Changes in NDF digestibility of this magnitude are huge and exceed those expected with substitution of brown midrib

corn silage for traditional corn silage. Oba and Allen (1999) suggested that a 1-percentage unit increase in NDF digestibility resulted in a 0.17 and 0.25 kg/d increases in DMI and 4.0% FCM, respectively. Using Oba and Allen (1999) coefficients and assuming a 7.5-percentage-unit increase in NDF digestibility by increasing DCAD from 0 to 500 mEq/kg, the expected increase in DMI and 3.5% FCM would be 1.3 and 1.9 kg/d, respectively and would account for 75% of the expected increase in DMI and 55% of the expected increase in 3.5% FCM. We concluded that one of the primary modes of action of DCAD is the increase in ruminal fluid pH and NDF digestibility.

Figure 1. Dry matter intake (A), milk production (B), milk fat percent (C), and milk fat yield (D) responses to increasing DCAD.

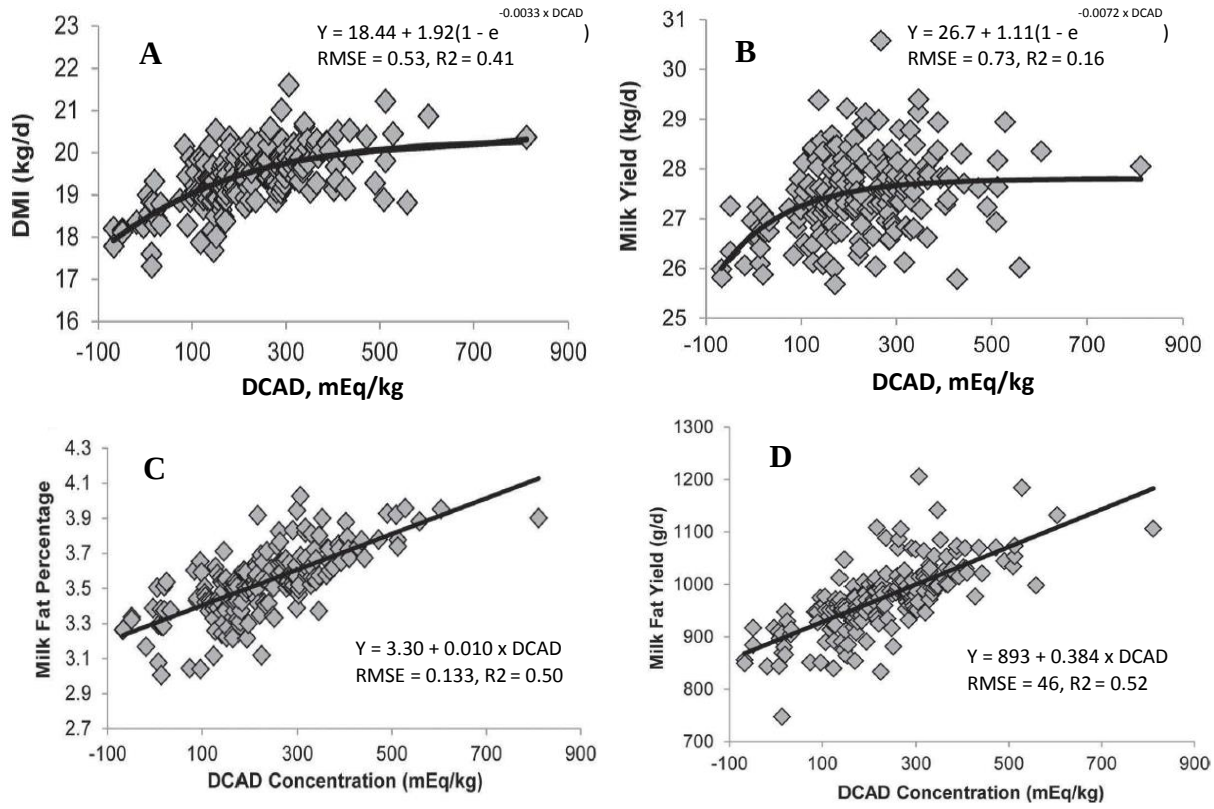
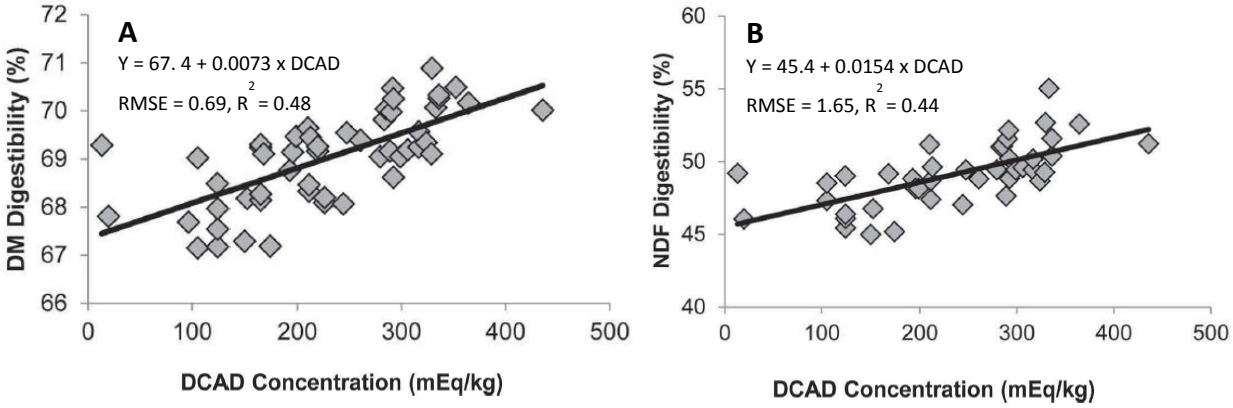


Figure 2. Effect of DCAD on digestibility of dry matter (DM) and NDF.



What is the Optimal DCAD for Lactating Dairy Cows?

As stated earlier, there is no NRC requirement for DCAD but feeding at the minimal requirements for Na, K, Cl, and S would result in a DCAD of 304 and 179 mEq/kg of dietary DM, respectively using the Mongin (1981) and Ender (1971) equations that differ by the incorporation of S in the DCAD calculation. **Table 5** shows a comparison of the maximum DMI, milk production, and FCM production responses from our summary (Iwaniuk and Erdman, 2015) and the earlier analysis of Hu and Murphy (2004). First, the primary economic response is milk fat yield which, in combination with a slight increase in milk production, drives increased FCM. Secondly, an optimal DCAD concentration is not necessarily the concentration at the maximal response. We prefer to look at DCAD concentrations somewhat below maximum because there is a cost in terms of added feed intake and addition of mineral supplements to increase DCAD. We view a practical minimum as a DCAD of 300 mEq/kg of dietary DM. This corresponds to two-thirds of the maximum response in DMI, will garner nearly all the added milk production, and achieve the majority of the increase in FCM production. After that point, the decision to feed higher DCAD with depend on the cost of supplementation and the added value of the extra milk fat produced.

Table 5. Comparisons of maximum responses to DCAD (Ender 1971 equation) from the analyses conducted by Iwaniuk and Erdman (2015) and those of Hu and Murphy (2004).

		66% of maximum	80% of maximum	Hu and Murphy (2004)
Item	Maximum response, kg/d	---- DCAD, mEq/kg of dietary DM required -----		
DMI	1.92	290	425	275
Milk	1.11	150	225	215
FCM	4.82	450	675	No maximum

Formulating for DCAD

Diet formulation for DCAD begins with feed ingredient selection. **Table 6** shows a comparison of selected feed ingredients and their relative mineral and DCAD concentrations. The first thing that is apparent is that most feeds have a relatively low Na content and vary substantially in K, and to a lesser extent, Cl and S. Therefore, feeds that are high in DCAD in which the cations (K and Na) are greater than the anions (Cl and S) are usually feeds that are high in K. Feeds like soybean meal, alfalfa haylage, barley, and grass silages are high K and also high DCAD. Corn silage, because it is a mixture of the corn plant (stalk and leaves) and grain, is intermediate in DCAD content. Protein supplements such as DDGS and canola meal are intermediate in K content and are low DCAD feeds because of their high S content. Thus, in selection of feed ingredients for high DCAD, you will normally look for feeds that are high in K content. Feeds like soybean meal and forages, especially alfalfa and small grain silages, will increase DCAD.

Generally, high NDF feeds (forages) are also high DCAD feeds because of their K content. One side benefit of increasing fiber (NDF) in the diet to increase milk fat is that this also indirectly increases DCAD. Dairy producers frequently attribute the increase in milk fat when NDF is increased to the added NDF, but part of the response is likely due to increased DCAD caused by substitution of low fiber and low DCAD feeds like corn for high fiber and high DCAD feeds like grass or small grain silages.

Table 6. Comparison of cation (K and Na), anion (Cl and S), and DCAD concentrations (mEq/kg of dietary DM) along with % crude protein (CP) and % NDF of feed ingredients.

Feed ingredient	K	Na	Cl	S	DCAD	CP, %	NDF, %
Shelled corn	107	9	-23	-63	31	9.4	9.5
DDGS	281	130	-28	-275	109	29.7	38.8
SBM	775	13	-155	-244	389	53.8	9.8
Canola meal	361	30	-11	-456	-76	37.8	29.8
Corn silage	307	4	-82	-88	142	8.8	45
Alfalfa haylage	775	13	-155	-188	445	22.8	36.3
Grass silage	795	22	-181	-131	505	18	49.9
Barley silage	621	57	-203	-106	369	12	56.3

Supplements That Can Be Used to Increase DCAD

There are a variety of Na and K carbonate and bicarbonate salts that can be used to raise DCAD once the inherent DCAD in feed ingredients has been accounted for. **Table 7** shows some commonly supplemented K and Na mineral salts used in dairy cattle diets. Please note that common salt (**NaCl**) and potassium chloride (**KCl**) are DCAD neutral since the cation (Na or K) is balanced by a corresponding anion (Cl). While salt and KCl are highly available sources of Na, K, and Cl, supplementing with these minerals will have no effect on DCAD. In order to raise DCAD, nutritionists must select from mineral supplements such as potassium carbonate, sodium bicarbonate, or sodium sesquicarbonate. Surprisingly, there is very little difference among these in their relative DCAD content (Table 7). Adding 0.75%, 0.83%, or 0.75% of commercially available potassium carbonate, sodium bicarbonate or sodium sesquicarbonate, respectively to dietary DM will increase DCAD by 100 mEq/kg dietary DM. At that point the choice of supplement is based on cost unless the minimum requirements for Na and K have not been met.

Conclusions

Dietary Anion Cation Difference is not only important for dry cows but also for lactating cows. Optimal DCAD for dry cow diets is typically zero or negative while feeding low DCAD diets to lactating cows will depress feed intake, milk production, and milk fat concentration. The minimal DCAD for lactating cows is most likely about 300 mEq/kg of feed DM (30 mEq/100 g of feed DM). However, the optimal DCAD will be dependent on the value of milk fat, which is the primary economic response to DCAD, and the cost of increasing DCAD above the diet's inherent DCAD concentration with mineral supplements.

Table 7. Composition of Na and K mineral supplements.

Mineral supplement	K, %	Na, %	Cl, %	DCAD, Eq/lb	DCAD, Eq/kg	DCAD
Salt (NaCl)	0.0	39.3	60.7	0	0	Neutral
Potassium chloride (KCl)	52.4	0.0	47.6	0	0	Neutral
Potassium carbonate (K_2CO_3)	52.4	0.0	0.0	609	1340	Positive
Sodium bicarbonate ($NaHCO_3$)	0.0	27.7	0.0	547	1203	Positive
Sodium sesquicarbonate ($Na_2CO_3 \cdot NaHCO_3 \cdot 2H_2O$)	0.0	30.5	0.0	602	1325	Positive

References

Block, E. 1984. Manipulating dietary anions and cations for prepartum dairy cows to reduce incidence of milk fever. J. Dairy Sci. 67: 2939–2948.

Goff, J. P., and R. L. Horst. 1997. Effects of the addition of potassium or sodium, but not calcium, to prepartum rations on milk fever in dairy cows. J. Dairy Sci. 80:176–186.

Dishington, I. W. 1975. Prevention of milk fever (hypocalcaemic paresis puerperalis) by dietary salt supplementation. Acta Vet. Scand. 16:503.

Ender, F., I. W. Dishington, and A. Helgebostad. 1971. Calcium balance studies in dairy cows under experimental induction and prevention of hypocalcaemic paresis puerperalis. Z. Tierphysiol. Tierernihr. Futtermittelkd. 28:233.

Erdman, R.A. 1988. Dietary buffering requirements of the lactating dairy cow: A review. J. Dairy Sci. 71: 3246–3266.

J.P. Goff, R. Ruiz, and R.L. Horst. 2004. Relative acidifying activity of anionic salts commonly used to prevent milk fever. J. Dairy Sci. 87: 1245–1255.

Hu, W. and M.R. Murphy. 2004. Dietary cation-anion difference effects on performance and acid-base status of lactating dairy cows: A meta-analysis. J. Dairy Sci. 87: 2222–2229.

Iwaniuk, M.E., and R.A. Erdman. 2015. Intake, milk production, ruminal, and feed efficiency responses to dietary cation-anion difference by lactating dairy cows. J. Dairy Sci. 98:8973–8985.

Mongin, P. 1981. Page 109 in Recent Advances in Animal Nutrition. W. Haresign, ed. Butterworths, London, Eng.

NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. Natl. Acad. Press, Washington, DC.

Oba, M. and M.S. Allen. 1999. Evaluation of the importance of digestibility of neutral detergent fiber from forage: Effects on dry matter intake and milk yield of dairy cows. J. Dairy Sci. 82: 589–596.

Sanchez, W.K. and D.K. Beede. 1996. Is there an optimal dietary cation-anion difference for lactation diets? Anim. Feed Sci. Technol. 59: 3–12.

W.B. Tucker, G.A. Harrison, and R.W. Hemken. 1988. Influence of dietary cation-anion balance on milk, blood, urine, and rumen fluid in lactating dairy cattle. J. Dairy Sci. 71:346–354.

West, J.W., K.D. Haydon, B.G. Mullinix, and T.G. Sandifer. 1992. Dietary cation anion balance and cation source effects on production and acid-base status of heat-stressed cows. J. Dairy Sci. 75: 2776–2786.

Impact of Starch Content and Digestibility in Dairy Cattle Diets

Luiz F. Ferraretto[1]
Department of Animal Sciences, University of Florida

Introduction

Compared with other nutrients, starch was the most under evaluated research topic in dairy nutrition for many years. Consequently, starch requirements for dairy cows were never established by the NRC (2001). Recently, improvements in the use of starch by lactating dairy cows garnered much interest by dairy farmers and their nutritionists, particularly over the past decade with the two-fold rise in corn prices. Consequently, starch utilization by lactating dairy cows became an important research topic. Thus, the objective of the present article is to present and discuss potential strategies to optimize starch utilization by lactating dairy cows.

Dietary Starch Content

High-producing dairy cows require high-energy diets to fulfill their genetic potential. Corn is the predominant energy source in the dairy industry with approximately 75% of the energy value in corn grain being contributed by starch. Therefore, the substantial increase in corn prices resulted in renewed interest in the potential for feeding reduced-starch diets. The Dairy NRC (2001) established energy but not starch requirements for dairy cows. Thus, other fermentable carbohydrates (i.e. fiber and sugars) may be fed to fulfill the established energy requirements of lactating dairy cows. Reduced-starch diets could be formulated by partially replacing corn grain with high-fiber, low-starch byproduct feedstuffs (e.g. soy hulls, citrus pulp, whole cottonseed, beet pulp, cottonseed hulls, and wheat middlings), high starch forages (i.e. whole-plant corn silage) or high-sugar ingredients (i.e. molasses, whey, and sucrose). Although these varied carbohydrate sources can be used for energy, their ruminal fermentation by microorganisms yields different fermentation end-products, which in turn alter metabolism and performance by dairy cows.

Starch is rapidly fermented by ruminal microorganisms into propionate. Propionate is absorbed into the bloodstream and transported to the liver, and later it is used as a precursor for glucose. If not digested in the rumen, starch reaches the small intestine and is digested by pancreatic amylase directly into glucose. Thus, despite starch not having established requirements, its supplementation directly affects glucose supply and thereby, lactation performance of dairy cows.

According to Shaver (2010), results from short-term (10 to 21 d) switchback feeding trials in the literature suggest that reduced-starch diets formulated by partially replacing corn grain with high-fiber, low-starch byproduct feedstuffs (e.g. soy hulls, citrus pulp, and whole cottonseed) may be feasible. However, few long-term (10 to 12 wk) feeding

[1] Contact: 2250 Shealy Drive, Gainesville, FL 32611. (352) 294-1005. Email: lferraretto@ufl.edu

trials can be found in the literature, and the effects of these feedstuffs on lactation performance should be verified in long-term continuous lactation trials. A summary of four continuous-lactation trials conducted at the University of Wisconsin – Madison was presented previously in this conference (Shaver, 2013). Across these four trials, feeding less dietary starch reduced actual-milk feed efficiency by 2 to 12% and solids-corrected milk feed efficiency by 1 to 11%. These results were related to either decreased milk production or increased DMI while maintaining similar milk production when starch was replaced with high-fiber, low-starch byproduct feedstuffs.

A recent review used a meta-analysis approach to evaluate the effect of dietary starch on lactation performance by dairy cows (Ferraretto et al., 2013). The authors considered only dietary starch values and not the specific type of carbohydrate used to replace starch. Starch concentration in the diet did not affect intake and this was thought to be related to two opposing effects: rumen fill limitation (Mertens, 1987) and increased ruminal propionate concentrations with corresponding decreased meal size (Allen et al., 2009) when corn grain was partially replaced by forage and non-forage fiber sources, respectively. Although milk yield increased 0.08 kg/d per %-unit increase in dietary starch content, feed conversion was unaffected by dietary starch. In addition, increased dietary starch concentration enhanced milk protein content. Reduced milk protein content for cows fed reduced-starch diets are related to lower starch intake reducing ruminal microbial protein production (Oba and Allen, 2003). Alternatively, less starch reaches the small intestine mediating milk protein content through alterations in arterial insulin concentrations (Rius et al., 2010). Conversely, milk fat content decreased as dietary starch content increased. Milk fat depression in high-starch diets is likely related to greater starch and lower NDF intakes (Jenkins and McGuire, 2006). The milk urea nitrogen concentration was also reduced by increasing dietary starch concentrations. Overall these data suggest better ruminal nitrogen utilization (NRC, 2001) as starch in the diet increases.

Another result highlighted by the meta-analysis of Ferraretto et al. (2013) is the effect of dietary starch concentration on in vivo NDF digestibility (**Figure 1**). The digestibility of dietary NDF decreased 0.61%-units ruminally and 0.48%-units total-tract per %-unit increase in dietary starch content. Similar to milk fat depression, decreased fiber digestibility may be partially explained by a decrease in ruminal fluid pH as a consequence of greater amounts of starch being digested in the rumen as starch intake increases. Low ruminal fluid pH is known to affect microbial growth and bacterial adherence and thereby fiber digestion. Also, the inherently high fiber digestibility of non-forage fibrous by-products used to partially replace corn grain in reduced-starch diets may be partly responsible. An exercise presented by Weiss (unpublished) during the 28th ADSA Discover Conference on Starch for Ruminants calculated the effects of a 0.5%-unit change in total tract NDF digestibility for each 1%-unit change in dietary starch content (slope of Figure 1) on dietary energy values. In the Weiss exercise, a 5%-unit increase in dietary starch content (e.g. 30% vs. 25%) would increase dietary NE_L content by 6.5% without accounting for adverse effects of dietary starch on total tract NDF digestibility. However, the reduction of 2.5-% units (46.5% to 44.0%) in total

tract NDF digestibility alters this scenario to a 5.3% increase in dietary NE_L content. Further incorporation of these effects on models are warranted.

Fredin (2015) conducted a meta-analysis to identify feeding strategies that could mitigate potential negative effects of feeding reduced-starch diets to lactating dairy cows. Milk yield was decreased when starch was replaced by either non-forage fiber sources (0.16 kg/d per %-unit decrease in dietary starch) or forage (0.32 kg/d per %-unit decrease in dietary starch). Reduced intake and ruminal degradation of forage NDF compared to non-forage NDF (Allen, 1997) were thought to induce greater reduction in milk yield when dietary starch was replaced by forage in the study by Fredin (2015). However, Fredin (2015) highlighted that 24 out of 61 treatment means for milk yield were greater for reduced-starch compared to high-starch diets, suggesting that positive lactation performance can be achieved when feeding reduced-starch diets. Yields of milk components were also reduced when dietary starch was replaced.

Potential negative effects on either milk yield or feed efficiency underscores that monitoring income over feed costs is recommended rather than price per unit of dietary DM to fully assess economic benefits of reduced-starch diets. Based on the summary of four continuous lactation trials (Shaver, 2013) and the meta-analysis reviews of literature (Ferraretto et al., 2013; Fredin, 2015), reducing dietary starch for peak and mid-lactation dairy cows may not be feasible and each scenario must be carefully evaluated.

Starch Digestibility in Corn Grain and Silage

The energy value of corn silage and grain contributed by starch is approximately 50 and 75%, respectively (calculated from NRC, 2001). Thus, to optimize starch availability in combination with the use of reduced-starch diets may have the potential to improve ruminal and total tract starch digestion. An increase in starch digestion may lead to better nutrient utilization and decreased feed costs. Detailed descriptions about factors influencing starch utilization in corn silage and grain will be discussed in this section.

Starch digestibility of whole-plant corn silage (**WPCS**), high-moisture corn (**HMC**) and dry ground corn (**DGC**) may be affected by several factors. First, the starch endosperm is protected by the pericarp which, if intact, is highly resistant to microbial attachment (McAllister et al., 1994); thereby breakage of the seed coat is obligatory. Diets containing HMC with mean particle size (**MPS**) below 2 mm had greater total tract starch digestibility (**TTSD**) compared with HMC with MPS greater than 2 mm (95.2% to 89.5%; Ferraretto et al., 2013). Likewise, increased MPS reduced TTSD in DGC-based diets (77.7% to 93.3% for 4 mm and 1 mm respectively; Ferraretto et al., 2013). This is related to increased surface area for bacterial and enzymatic digestion of finer particles (Huntington, 1997). Greater starch digestibility and corresponding milk production by dairy cows is achieved when corn silage is harvested using a kernel processor with roll gap settings between 1 to 3 mm (Ferraretto and Shaver, 2012). However, other harvesting practices may impair the efficacy of kernel processors.

Kernel processing was effective when theoretical length of cut (**TLOC**) settings on choppers was set at 0.93 to 2.86 cm but not when set at shorter or longer settings (Ferraretto and Shaver, 2012). This could be possibly explained by greater kernel breakage by cutting knives at the lower TLOC (Johnson et al., 1999) or inhibition of kernel breakage during passage through the rollers by the stover portion at the longer TLOC. Furthermore, processing increased TTSD for diets containing WPCS with 32% to 40% DM at feed-out, but not when WPCS was above 40% DM (Ferraretto and Shaver, 2012). An increased proportion of vitreous endosperm in the kernel is associated with greater maturity (Phillipeau and Michalet-Doureau, 1997). Increased kernel vitreous endosperm increases kernel hardness which in turn may cause kernels in very dry corn silage to be less susceptible to breakage during kernel processing at harvest.

Even the exposed endosperm is not fully digested due to existence of a starch-protein matrix formed by the chemical bonds of zein proteins with starch granules (Kotarski et al., 1992; McAllister et al., 1993). Ruminal in vitro starch digestibility was greater when HMC was harvested at lower DM content (**Figure 3**; Ferraretto et al., 2014). Furthermore, reduced TTSD were detected in diets containing WPCS above 40% DM in the meta-analysis review by Ferraretto and Shaver (2012). This may be related to an increase in the proportion of vitreous endosperm in the kernel associated with greater maturity (Correa et al., 2002; Ngonyamo-Majee et al., 2009). Alternatively, a reduction in the extent of fermentation for drier WPCS (Der Bedrosian et al., 2012) may attenuate the breakdown of zein proteins during fermentation (Hoffman et al., 2011). Goodrich et al. (1975) harvested HMC with 67% DM and oven-dried corn to 73% and 79% DM to study the effects of moisture content on fermentation of HMC. They reported a decrease in acetate and lactate concentrations and a corresponding increase in pH as DM content of HMC increased. Lower lactate and acetate concentrations are likely related to a reduced bacterial growth due to limited water availability (Muck, 1988). Goodrich et al. (1975) also observed reduced ruminal in vitro gas production as DM content increased, suggesting reduced starch digestibility for HMC at greater DM contents. These results combined suggest that proper maturity at harvest is required to maximize starch digestibility in WPCS and HMC.

Research trials on the effects of ensiling time on ruminal in vitro starch digestibility (**ivSD**) of WPCS are summarized in **Table 1**. At 30 or 45 days of ensiling, starch digestibility was increased by 7 percentage units on average and is likely related to the fermentation phase which typically occurs in this time frame. Interestingly, all 7 trials had a gradual increase in ivSD after additional storage time suggesting that perhaps ivSD continuously increases during storage. Proteolytic activity, either from microbial or plant proteases, occurs more extensively during the anaerobic fermentation process (Baron et al., 1986). The anaerobic phase is characterized by a drastic decrease in pH (Muck, 2010) which favors the activity of plant proteases specific to the endosperm of cereal grains (Simpson, 2001), even though the activity of plant proteases is typically reduced under low pH (Muck, 1988). Junges et al. (2015) evaluated the contribution of proteolytic sources on protein solubilization in rehydrated corn ensiled for 90 d. These authors reported that bacterial proteases are responsible for 60% of the increase in

soluble CP concentration, followed by kernel enzymes (30%), and fungi and fermentation end-products (5% each).

Although allowing an extended ensiling period may be beneficial for increasing starch digestibility in situations where coarser, drier, or more vitreous hybrids are harvested, research in this area is still limited. Two other studies (Ferraretto et al., 2015a,b) were conducted to evaluate the interaction between hybrid types and ensiling time on starch digestibility of WPCS. Our hypothesis was that prolonged storage would attenuate, or perhaps overcome, the difference in starch digestibility between hybrid types. In the first experiment (Ferraretto et al., 2015b), another industry-university collaborative study, 8 WPCS hybrids (4 bm_3 and 4 leafy) were ensiled for 0, 30, 120, and 240 d. Although ivSD was similar between hybrids throughout the storage period, the N fraction response to time of fermentation varied with hybrid type suggesting greater effects on the breakdown of zein proteins in leafy than bm_3 hybrids. The second experiment (Ferraretto et al., 2015a) compared 3 hybrids (bm_3, dual-purpose, and experimental floury-leafy) ensiled for 0, 30, 60, 120, and 240 d. Contrary to our hypothesis, however, extended ensiling time did not attenuate the negative effects of kernel vitreousness on ivSD. The results from these experiments emphasize the importance of further WPCS starch digestibility research with regard to potential interactions between hybrid, harvest maturity, kernel processing, and ensiling. Furthermore, results suggest that the best opportunity for benefit from altering kernel endosperm properties for greater starch digestibility may reside within the bm_3 type hybrids.

On-Farm Assessment of Starch Digestibility

Fredin et al. (2014) reported a strong relationship between fecal starch measurements and TTSD. These results suggest that additional measurements to fecal starch, such as starch content of the diet or indigestible marker concentrations (iNDF or lignin) in the feces or diet are unnecessary. Furthermore, Fredin et al. (2014) reported high accuracy of near infrared reflectance spectroscopy (**NIRS**) to predict fecal starch, which allows for more rapid and inexpensive analysis. Although benefits of greater starch digestibility on milk production is well known, it is very difficult to reliably estimate its economic impact. The exercise presented and discussed in this article is an attempt to provide some numbers to dairy producers and their nutritionists as a starting point.

To accomplish our goal, a hypothetical scenario was created and five values of fecal starch were arbitrarily chosen and used to predict TTSD using the equation of Fredin et al. (2014; **Table 2**). Subsequently, the amount of corn that would need to be supplemented in order to obtain the same amount of digestible starch as if TTSD was 100% was estimated using the following assumptions: dietary starch was 25% of DM and consumption of DM was 55 lbs/d. Consequently, it was assumed that cows were eating 13.75 lbs of starch per day. Based on TTSD, values of starch loss in the manure was calculated and ranged from 0 to 3.5 lbs. If one consider that corn grain has 70% starch and 70% ruminal in vitro starch digestibility, for each lb of corn supplemented

only 0.49 lbs of digestible starch is provided. Thus, by dividing starch loss by 0.49 we reached the amount of corn necessary to fulfill for undigested starch. Last, US$130.40/ton (approximately US$0.065/lb) was used to calculate corn grain costs. Values used in the present exercise is not representative of the entire American dairy industry, but it is a good indication of potential economic loss related to low starch digestibility. Thus, it is recommended that dairy farmers and their nutritionists perform similar calculations based on their own scenarios and goals.

Summary

Fecal starch does not indicate digestibility of specific feedstuffs but of total diets, and it can be used as a valuable tool to monitor specific groups of cows over time by collecting samples from at least 10% of animals in the group. If fecal starch levels are above 3%, specific starchy feedstuffs should be evaluated to elucidate the problem. In addition, re-evaluation of fecal starch values are recommended after 2 or 3 weeks of dietary or management adjustments.

References

Allen, M. S., B. J. Bradford and M. Oba. 2009. The hepatic oxidation theory of the control of feed intake and its application to ruminants. J. Anim. Sci. 87:3317-3334.

Baron, V. S., K. R. Stevenson, and J. G. Buchanan-Smith. 1986. Proteolysis and fermentation of corn-grain ensiled at several moisture levels and under several simulated storage methods. Can. J. Anim. Sci. 66:451-461.

Correa, C. E. S., R. D. Shaver, M. N. Pereira, J. G. Lauer, and K. Kohn. 2002. Relationship between corn vitreousness and ruminal in situ starch degradability. J. Dairy Sci. 85:3008-3012.

Der Bedrosian, M. C., L. Kung, Jr., and K. E. Nestor, Jr. 2012. The effects of hybrid, maturity and length of storage on the composition and nutritive value of corn silage. J. Dairy Sci. 95:5115-5126.

Ferraretto, L. F., P. M. Crump, and R. D. Shaver. 2013. Effect of cereal grain type and corn grain harvesting and processing methods on intake, digestion and milk production by dairy cows through a meta-analysis. J. Dairy Sci. 96:533-550.

Ferraretto, L. F., P. M. Crump, and R. D. Shaver. 2015a. Effect of ensiling time and exogenous protease addition to whole-plant corn silage of various hybrids, maturities and chop lengths on nitrogen fractions and ruminal in vitro starch digestibility. J. Dairy Sci. 98:8869-8881.

Ferraretto, L. F., S. M. Fredin, R. E. Muck and R. D. Shaver. 2016. Microbial inoculant and ensiling time effects on fermentation profile, nitrogen fractions, and ruminal in vitro and in situ starch digestibility in corn shredlage and late-maturity-corn silage. Prof. Anim. Sci. 32:861-868.

Ferraretto, L. F., and R. D. Shaver. 2012. Meta-analysis: Impact of corn silage harvest practices on intake, digestion and milk production by dairy cows. The Prof. Anim. Sci. 28:141-149.

Ferraretto, L. F., R. D. Shaver, S. Massie, R. Singo, D. M. Taysom, and J. P. Brouillette. 2015b. Effect of ensiling time and hybrid type on fermentation profile, nitrogen

fractions and ruminal in vitro starch and NDF digestibility in whole-plant corn silage. The Prof. Anim. Sci. 31:146-152.
Ferraretto, L. F., K. Taysom, D. M. Taysom, R. D. Shaver, and P. C. Hoffman. 2014. Relationships between dry matter content, ensiling, ammonia-nitrogen, and ruminal in vitro starch digestibility in high-moisture corn samples. J. Dairy Sci. 97:3221-3227.
Fredin, S. 2015. How to get more out of dietary starch and low starch diets. Pages 91-101 in Proc. 77th Cornell Nutrition Conference for Feed Manufacturers. Ithaca, NY.
Fredin, S. M., L. F. Ferraretto, M. S. Akins, P. C. Hoffman, and R. D. Shaver. 2014. Fecal starch as an indicator of total tract starch digestibility by lactating dairy cows. J. Dairy Sci. 97:1862-1871.
Goodrich, R. D., F. M. Byers, and J. C. Meiske. 1975. Influence of moisture content, processing and reconstitution on the fermentation of corn grain. J. Anim. Sci. 41:876-881.
Hoffman, P. C., N. M. Esser, R. D. Shaver, W. K. Coblentz, M. P. Scott, A. L. Bodnar, R J. Schmidt, and R. C. Charley. 2011. Influence of ensiling time and inoculation on alteration of the starch-protein matrix in high-moisture corn. J. Dairy Sci. 94:2465-2474.
Huntington, G. B. 1997. Starch utilization by ruminants: From basics to the bunk. J. Anim. Sci. 75:852-867.
Jenkins, T. C., and M. A. McGuire. 2006. Major advances in nutrition: impact on milk composition. J. Dairy Sci. 89:1302-1310.
Johnson, L., J. H. Harrison, C. Hunt, K. Shinners, C. G. Doggett, and D. Sapienza. 1999. Nutritive value of corn silage as affected by maturity and mechanical processing: A contemporary review. J. Dairy Sci. 82:2813-2825.
Junges, D., G. Morais, J. L. P. Daniel, M. H. F. Spoto, and L. G. Nussio. 2015. Contribution of proteolytic sources during fermentation of reconstituted corn grain silages. In: XVII International Silage Conference, Piracicaba, SP, Brazil. Pages 566-567.
Kotarski, S. F., R. D. Waniska, and K. K. Thurn. 1992. Starch hydrolysis by the ruminal microflora. J. Nutr. 122:178-190.
McAllister, T. A., H. D. Bae, G. A. Jones and K. J. Cheng. 1994. Microbial attachment and feed digestion in the rumen. J. Anim. Sci. 72:3004-3018.
McAllister, T. A., R. C. Phillippe, L. M. Rode, and K. J. Cheng. 1993. Effect of the protein matrix on the digestion of cereal grains by ruminal microorganisms. J. Anim. Sci. 71:205-212.
Mertens. D. R. 1987. Predicting intake and digestibility using mathematical models of ruminal function. J. Anim. Sci. 64:1548-1558.
Muck, R. E. 1988. Factors affecting silage quality and their implications for management. J. Dairy Sci. 71:2992-3002.
Muck, R. E. 2010. Silage microbiology and its control through additives. R. Bras. Zootec. 39:183-191 (Supl. Especial)
Ngonyamo-Majee, D., R. D. Shaver, J. G. Coors, D. Sapienza, and J. G. Lauer. 2009. Influence of single-gene mutations, harvest maturity and sample processing on ruminal in situ and post-ruminal in vitro dry matter and starch degradability of corn grain by ruminants. Anim. Feed Sci. Technol. 151:240-259.

NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Sci., Washington, D.C.
Oba, M. and M. S. Allen. 2003. Effects of corn grain conservation method on ruminal digestion kinetics for lactating dairy cows at two dietary starch concentrations. J. Dairy Sci. 86:184-194.
Philippeau, C., and B. Michalet-Doreau. 1997. Influence of genotype and stage of maturity of maize on rate of ruminal starch degradation. Anim. Feed Sci. Technol. 68:25-35.
Rius, A.G ., J. A. D. R. N. Appuhamy, J. Cyriac, D. Kirovski, O. Becvar, J. Escobar, M. L. McGilliard, B. J. Bequette, R. M. Akers, and M. D. Hanigan. 2010. Regulation of protein synthesis in mammary glands of lactating dairy cows by starch and amino acids. J. Dairy Sci. 93:3114-3127.
Shaver, R. 2013. Alternatives to corn for dairy rations. Pages 98-105 in Proc. 24th Florida Ruminant Nutrition Symposium. Gainesville, FL.
Shaver, R. 2010. Improving starch digestibility in dairy cows: Opportunities with reduced-starch diets. Pages 90-93 in Proc. Four-State Dairy Nutr. & Mgmt Conf. Dubuque, IA.
Simpson, D. J. 2001. Proteolytic degradation of cereal-prolamins – The problem with proline. Plant Sci. 161:825-838.
Windle, M. C., N. Walker, and L. Kung, Jr. 2014. Effects of an exogenous protease on the fermentation and nutritive value of corn silage harvested at different dry matter contents and ensiled for various lengths of time. J. Dairy Sci. 97:3053-3060.
Young, K. M., J. M. Lim, M. C. Der Bedrosian, and L. Kung, Jr. 2012. Effect of exogenous protease enzymes on the fermentation and nutritive value of corn silage. J. Dairy Sci. 95:6687-6694.

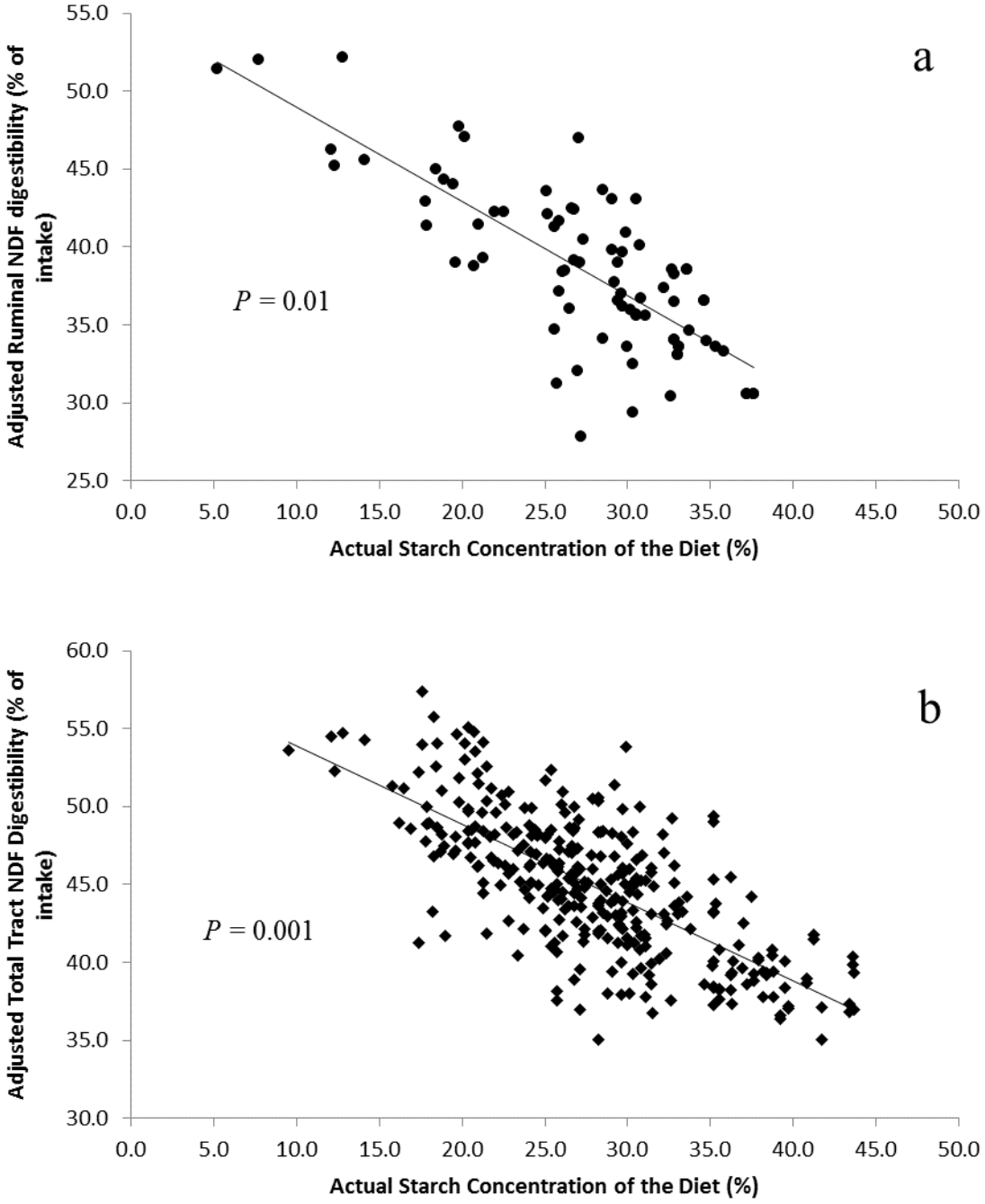


Figure 1. Effect of starch concentration of the diet on ruminal and total-tract digestibility of diet NDF adjusted for the random effect of trial. Ruminal digestibility data (Panel a) predicted from equation: y = 54.9746 + (-0.605*starch concentration) + (0.063 ± 3.524); n = 70, RMSE = 3.55. Total-tract digestibility diet (Panel b) predicted from equation: y = 58.2843 + (-0.4817*starch concentration) + (0.059 ± 3.191); n = 320, RMSE = 3.20. Source: Ferraretto et al., 2013.

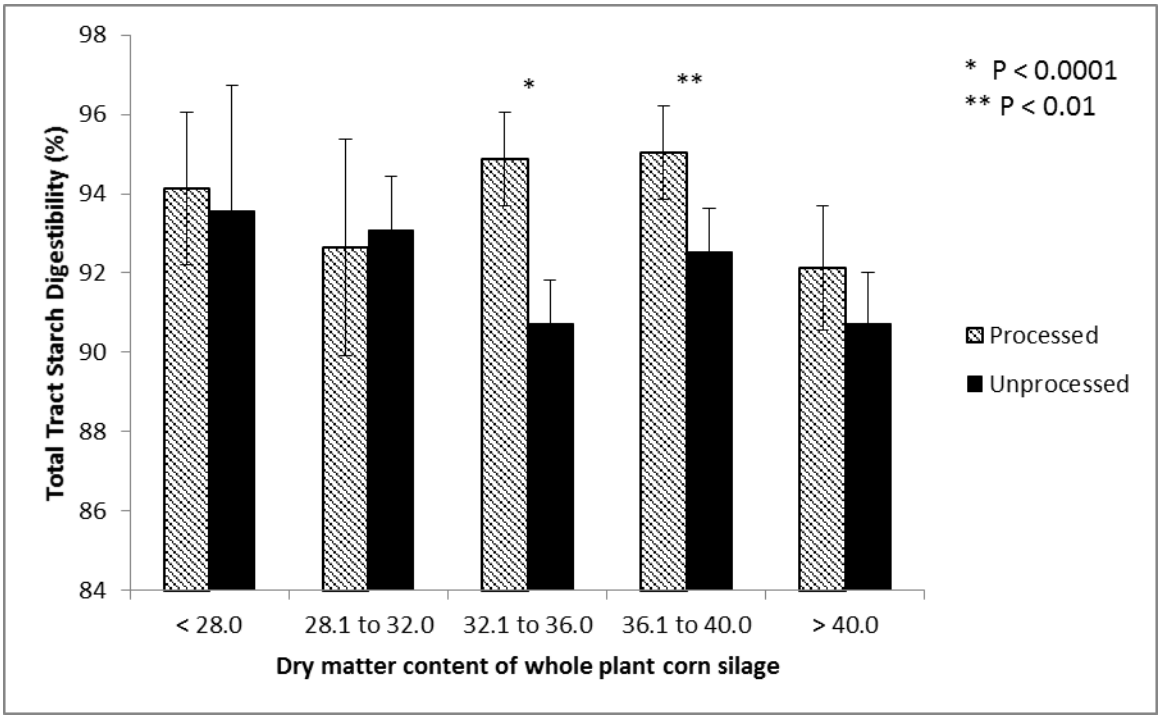


Figure 2. Effect of kernel processing and dry matter content of whole plant corn silage on total tract digestibility of dietary starch. Source: Ferraretto and Shaver (2012).

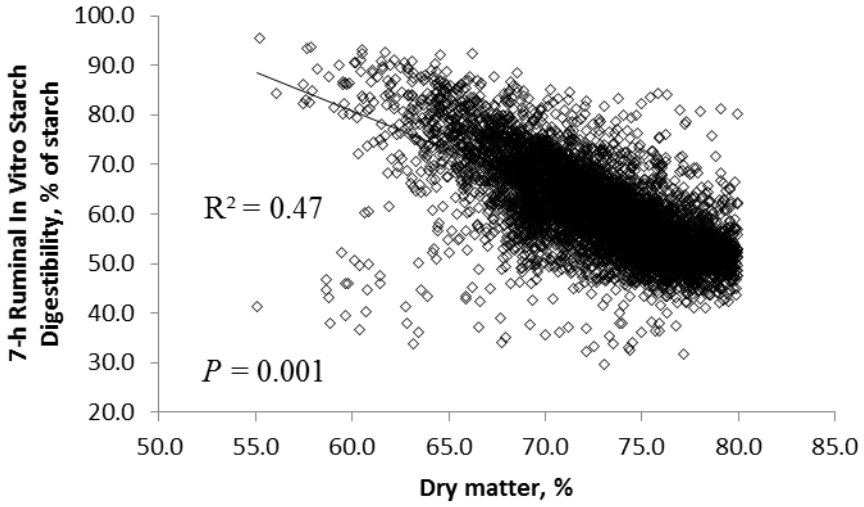


Figure 3. Relationship between DM content and 7-h ruminal in vitro starch digestibility in high moisture corn. Predictive equation: y = 174.30 (± 1.57) - 1.56x (± 0.02); n = 6,131, RMSE = 6.97, R^2 = 0.47, *P* = 0.001. Source: Ferraretto et al. (2014).

Table 1. Effects of ensiling time on ruminal in vitro starch digestibility in whole-plant corn silage[1]

	Days ensiled											
Reference	0	30	45	60	90	120	150	180	240	270	360	*P*-value
Whole-plant corn silage	- % of starch - - - - - - - - - - - - - - - - - - -											
Der Bedrosian et al., 2012[1]	69	---	75	---	77	---	---	79	---	82	82	0.01
Windle et al., 2014[1]	54	---	59	---	63	---	68	---	---	---	---	0.01
Young et al., 2012[1]	66	---	76	---	---	---	79	---	---	---	---	0.01
Ferraretto et al., 2015a[2]	56	59	---	61	---	63	---	---	67	---	---	0.01
Ferraretto et al., 2015b[2]	62	72	---	---	---	79	---	---	84	---	---	0.01
Ferraretto et al., 2016 – exp. 1[2]	60.7	69.3	---	---	---	72.0	---	---	---	---	---	0.05
Ferraretto et al., 2016 – exp. 2[2]	54.0	61.7	---	---	---	66.7	---	---	---	---	---	0.04

[1,2] Ruminal in vitro starch digestibility at 7 h on samples ground through a 3-mm or 4-mm screen, respectively.

Table 2. Economic estimates of corn supplemented to fulfill undigested starch.

Measure	Fecal starch, % of DM				
	0	5	10	15	20
TTSD[1], % of starch	100	93.75	87.50	81.25	75.00
Starch intake[2], lbs/cow per day	13.75	13.75	13.75	13.75	13.75
Starch loss[3], lbs/cow day	0	0.89	1.72	2.58	3.44
Corn grain supplementation[4], lbs/cow per day	0	1.82	3.51	5.37	7.02
Corn grain cost[5], US$/cow per day	0.00	0.12	0.23	0.35	0.46

[1] Predicted from equation of Fredin et al. (2014); Total Tract Starch Digestibility (TTSD) = 100 – (1.25 x fecal starch).
[2] Starch intake = (55 lbs DMI x 25% starch) / 100.
[3] Starch loss = starch intake – ((starch intake x TTSD) / 100).
[4] Corn grain supplementation = starch loss / 0.49.
[5] Corn grain cost = corn grain supplementation x 0.0652. Corn grain cost obtained from values reported by FeedVal 2012 on November, 2016.

Predicting Forage Intake by Grazing Beef Cows[2]

Stacey A. Gunter, PAS[3]
USDA-ARS Southern Plains Range Research Station, Woodward, OK 73801

Introduction

The control of feed intake by ruminants is complex, and developing a cohesive theory of intake control in ruminants continues to be a challenge. Because our understanding of factors that regulate intake by cattle is inadequate, predicting feed intake, even under the best of circumstances, is difficult. In grazing cattle, this difficulty is exacerbated by additional influences that can sway basic control mechanisms, including selective grazing, herbage mass, sward structure and composition, climatic and environmental factors, and the intricacies of the grazing process itself.

The sheer complexity of intake control in ruminants and the associated lack of mechanistic models has led to a reliance on empirical approaches. Fisher (2002) suggested that empirical models, despite their frequent lack of intellectual elegance, have considerable merit leading to many practical applications in beef cattle feeding. Generally, most empirical models in use today are based on the physical/physio-chemical theory of intake regulation. Thus, intake of less digestible, low-energy diets is mostly controlled by physical factors like ruminal fill and digesta passage where intake of highly digestible, high-energy diets is mostly controlled by energy demands of the animal and by metabolic factors (e.g., ruminal acidity and metabolic protein yield; NRC (1985, 1987). Examples of empirical equations that reflect the role of energy concentration in controlling feed intake are those based on body weight (**BW**) and dietary net energy for maintenance (**NEm**) concentration recommended by the NASEM (2016) beef nutrient requirements publication.

Regardless of their composition, empirical equations for predicting intake are far from perfect, typically accounting for only 50 to 70% of the variation in intake, with relatively high standard errors of prediction (5% of the mean or greater) where intake was measured directly. When applied to grazing situations, these equations might yield less than desirable accuracy and precision. In this review, I will summarize some of the factors that affect intake by grazing cattle and current means of predicting intake.

---

[2] Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA. The USDA prohibits discrimination in all its programs and activities on the basis of race, color, national origin, age, disability, and where applicable, sex, marital status, familial status, parental status, religion, sexual orientation, genetic information, political beliefs, reprisal, or because all or part of an individual's income is derived from any public assistance program. USDA is an equal opportunity provider and employer.

[3] Corresponding author: stacey.gunter@ars.usda.gov

Factors Affecting Grazed Forage Intake

Factors Affecting Selective Grazing

Total mixed rations (**TMR**) are often used in penned cattle in an effort to provide a uniform supply of nutrients, but sorting of dietary components from TMR is well-documented (Leonardi and Armentano, 2003). Thus, ruminants are inherently driven to select for certain types and sizes of feed components, thereby modifying their nutrient intake relative to the composition of the feed offered. For grazing cattle, the pasture resource is constantly changing. Preferred plant parts from the grazing domain have been removed after a meal, and the pasture has become more or less mature depending on the temporal scale. Hence, grazing cattle potentially have a different forage resource from which to select their diet each day, potentially affecting intake in a variety of ways.

The interaction of cattle with their landscape can be separated into space and time scales. Scale is a required concept in grazing ecology research and model building that would predict voluntary intake, referring primarily to the spatial and temporal dimensions at which cattle are observed (**Figure 1**). For example, estimates of grazed forage intake are often measured in relatively small pastures (e.g. 7 to 49 ha) and for short periods of time (e.g., 5 d; Krysl et al., 1987; Gunter et al., 1997). Nonetheless, data collected at these limited scales are often applied over weeks or months (greater temporal scales) and in extensive grazing environments of much greater spatial scale. More often than not, the empirical models that are used to make management decisions were constructed with cattle that were sensing and processing information at a different spatiotemporal scale than the cattle to which the models are applied. Hence, patterns and processes observed in animal behavior, including forage intake, depend greatly on the scale at which they were studied (Senft et al., 1987).

Differences noted in dietary quality and the voluntary intake by grazing cattle are associated with individual animal preferences and choices. The decisions to eat and which foods to consume are made on the basis of the expected reward and are influenced by past experiences, which then influences the animal's "wanting" and "liking" for food (Provenza et al., 2015a). Ginane et al. (2015) asserted that wanting, liking, and learning are different aspects of the food-selection process. Wanting is the motivation for the reward, which might be initiated by the internal state of the animal or by external stimuli. Liking is the pleasure component of a reward, which encompasses conscious and unconscious responses. Learning by cattle is associated with a past reward based on experiences. The positive attributes of learning are especially noticeable when new cows are introduced to a novel grassland, which sometimes results in a high percentage of the cows having difficulty maintaining body condition score (**BCS**) compared with experienced cohorts.

Choices of food by cattle span generations and the expression of these choices is influenced during critical periods of fetal development, which can have influences on life-long feeding behaviors. Villalba et al. (2015) showed how experiences in utero and

early life can cause physiological changes that alter food preferences and voluntary forage intake later in life. By interacting with the genome during growth and development, environments influence gene expression and behavioral responses (Provenza et al., 2015b). For example, lambs exposed to saltbush (*Atriplex* spp.) in utero, grew faster and handled greater salt loads than lambs gestated in ewes grazing mono-cultures of introduced grasses (Chadwick et al., 2009). Sheep (Distel et al., 1994) and cattle (Wiedmeier et al., 2012) exposed early in life to forages high in fiber had increased nitrogen retention and the ability to digest fiber more completely later in life than cohorts reared in utero on low-fiber diets. Based on these and other experiments, the “absolute value” of a food can change because the ability of animals to utilize forages can be enhanced or diminished by developmental experiences in utero. Hence, each grazer could potentially select a different diet depending on its learned and genetic preferences, a phenomenon that is extremely difficult to empirically model.

Part of the reason for selective grazing is that livestock attempt to maintain a balance between energy and protein in their diets, a balance that is achieved by associating the flavors of foods with nutrient-specific feedbacks. For example, lambs fed diets low in energy or protein preferentially ate non-nutritive flavored food previously associated with the feedback from ruminal infusions of protein or energy, respectively (Villalba and Provenza, 1996). Moreover, lambs chose a diet that would maximize growth when offered isocaloric foods that varied in protein and they ate less protein as they aged, reflecting a decreased requirement (Kyriazakis and Oldham, 1993). Forage intake decreases with imbalances of energy relative to protein and increases with appropriate ratios of energy to protein. When sheep are fed protein- or energy-imbalanced diets, they will then graze in locations with forages that rectify the nutrient imbalances (Scott and Provenza, 2000). In addition, steers (BW = 301 ± 26 kg) grazing a low-protein native prairie and fed 500 g/d of a 32% CP supplement, selected 76% fewer forbs than non-supplemented cattle to maintain a favorable protein to energy balance (Odadi et al., 2013). Thus, ruminants seem to sense dietary protein content and modulate short-term intake of flavored foods, seeking additional protein to balance their protein-to-energy intake ratio, ultimately affecting total voluntary intake.

Effects of ambient temperature on feed intake, digestibility, and rate of passage of pen-fed ruminants have been studied extensively and reviews are available on the subject (Kennedy et al., 1986). Fewer data are available for grazing ruminants, but effects are likely similar between pen-fed and grazing cattle in terms of the physiological consequences of heat and cold stress. In experiments with controlled environmental conditions, it seems clear that feed intake increases when the temperature falls below the lower point of the thermoneutral zone (generally -15 to 28^{o}C for mature beef cows; FASS, 2010) and decreases as the ambient temperature rises above the upper point (NRC, 1987). Ruminal motility and passage rate of digesta increase before changes in intake are observed under cold stress conditions, which led Kennedy et al. (1986) to suggest that these responses could be fundamental to the eventual increase in feed intake observed with cold stress.

Predicting Intake

Inherent Variability in Feed Intake and How it Affects Strategies for Prediction

Anyone who has ever fed cattle individually and plotted their daily intakes knows that intake by an individual animal is naturally variable, even with forage-based diets. Forbes (2003) plotted such data for a beef steer fed grass silage ad libitum with a daily allotment of 3 kg of a concentrate feed (**Figure 2**). The pattern of intake was similar to what might be expected with randomly generated data based on the same mean and standard deviation as the observed data. Some evidence generated through examining correlations among days indicated that the variability might reflect a pattern in which intake was responding in a 3- to 4-day cycle, but further experimental work and analyses would be needed to assess that idea. Forbes (2003) used these observations to suggest that this variability in feed intake was related to a control mechanism in which the animal adjusts its intake from day to day in response to discomfort signals. Assuming that this type of pattern, with daily or short-term intake varying considerably over time, very likely occurs in grazing cattle, it is appropriate to question how this variability might affect the measurement of grazed forage intake.

Potential Methods of Predicting Intake by Grazing Ruminants

National Academy of Science, Engineering, and Medicine Equations. The NASEM (2016) provided equations to predict intake by both growing-finishing beef cattle and beef cows. To develop the equation for growing-finishing cattle, published data from experiments conducted from 1980 to 1992 were summarized to yield 185 data points. Values represented average dry matter intake (**DMI**) for periods that varied from 56 to 212 d. Measurements of initial and final BW, information on whether the cattle were fed an ionophore or received a growth-promoting implant (approximately half the cattle), and descriptive information on frame size, gender (steer, heifer, or bull), age (calf or yearling), and initial and final BW were recorded. The NEm concentration of the diets (calculated from tabular values or actually determined in the study) was used to calculate total NEm intake as the product of dietary NEm concentration and DMI, and total NEm intake was scaled to a metabolic BW (**MBW**) basis (using the average $BW^{0.75}$ in kg). The relationship between NEm/MBW and dietary NEm concentration was established by stepwise regression analysis, which accounted for approximately 70% of the variation in NEm/MBW in the literature dataset. The intercept differed between calves vs. yearlings, yielding the following equations:

Calves: NEm intake, Mcal/d = $BW^{0.75}$ x (0.2435 x NEm – 0.0466 x NE_m^2 – 0.1128);
Yearlings: NEm intake, Mcal/d = $BW^{0.75}$ x (0.2435 x NEm – 0.0466 x NE_m^2 – 0.0869);

where BW is the average BW ([initial BW + final BW]/2) for a feeding period, and NEm is the dietary NEm concentration (Mcal/kg of DM). Dry matter intake (kg/d) is calculated from these equations by dividing total NEm intake predicted by the equations by dietary NEm concentration. The NASEM (2016) recommended that the divisor to determine

DMI from these equations be set to 0.95 for diets with NEm concentrations of ≤ 0.95 Mcal/kg of DM.

To predict intake by beef cows, the NASEM (2016) used a similar approach to equation development that was used for growing-finishing beef cattle. Treatment means were compiled from published articles, as well as unpublished theses and data from individual scientists, resulting in 153 observations for DMI (average for a feeding period; 21 to > 200 d) by non-pregnant beef cows or by cows during the middle and last third of pregnancy. Total NE_m intake/MBW was predicted from dietary NEm concentration, resulting in the following equation:

$$\text{NEm intake, Mcal/d} = BW^{0.75} \times (0.04997 \times NEm^2 + 0.04631);$$

the intercept for non-pregnant cows is 0.03840.

As with the growing-finishing beef cattle equation, DMI is calculated by dividing the predicted total NEm intake (Mcal/d) by the dietary NEm concentration (Mcal/kg of DM). Likewise, for low-quality forages with NEm concentrations of less than 1 Mcal/kg (approximately 50% TDN), the divisor should be set at 0.95. Finally, for lactating cows, NASEM (2016) suggested that predicted DMI be increased by a factor of 0.2 × the daily milk production (kg) and also advised users that the equation was probably not applicable for predicting DMI with protein-deficient forages.

Although the growing-finishing and beef cow equations of NASEM (2016) have been used extensively in practice, concerns have been expressed about prediction errors with both equations (Anele et al., 2014; Coleman et al., 2014).

The most unique models presented by Coleman et al. (2014) are the equations for lactating cows. The best measures of a cow's performance are her ability to rebreed and her calf production, particularly weaning weight. The direct nutritional output from the cow to the calf is milk. Milk production was a positive driver for cow voluntary organic matter intake (**OMI**), accounting for 56% of the variation in adjusted intake. The overall equation that Coleman et al. (2014) presented was:

$$\text{OMI (kg/d)} = 71.6 + 0.015 \times BW - 2.4D + 0.021 \times D^2 - 11.7 \times MP + 0.42 \times MP \times D - 0.0036 \times MP \times D^2;$$

where MP = milk production (kg/d) and D = digestibility (% of organic matter). Lactation causes the gastrointestinal tract to increase in size (Forbes, 1986) and increases voluntary OMI (NASEM, 2016) compared with non-lactating cows, regardless of pregnancy status. Nonetheless, milk production is difficult to measure in production environments. Therefore, including milk production in a general intake prediction equation makes little sense when managers will not likely have these data available.

Two possible surrogates for milk production are calf average daily gain (**ADG**) or calf weaning weight. After examining their data, Coleman et al. (2014) noted that calf ADG is more closely related to milk production. Calf pre-weaning ADG was a good

predictor of OMI and explained 64% of the variation when combined with BW and digestibility (**Figure 3**), which is a better predictor than milk production. The following equation describes the overall relationship presented by Coleman et al. (2014):

$$OMI\ (kg/d) = 251 - 0.06 \times BW + 0.00008 \times BW^2 - 7.6D + 0.062 \times D^2 - 265 \times G + 8.7 \times G \times D - 0.07 \times G \times D^2;$$

where W = cow BW (kg), D = digestibility (% of organic matter), and G = calf pre-weaning ADG (kg). Thus, calf performance seems to be a good integrator of cow intake by combining cow size and calf growth potential with milk production. Thus, it is logical that calf performance, measured as either weaning weight or ADG, might be more closely related to intake demand than milk production. On the basis of simple statistics (R^2 and residual SE), calf weaning weight was not as good an independent variable as pre-weaning ADG for predicting OMI by cows (Coleman et al., 2014), but the equation is included below because calf weaning weight is probably the easiest metric to estimate:

$$OMI\ (kg) = 266 - 0.08 \times W + 0.00009 \times W^2 - 8.1 \times D + 0.067 \times D^2 - 1.06 \times WW + 0.036 \times WW \times D - 0.00029 \times WW \times D^2;$$

where W = cow BW (kg), D = digestibility (% of organic matter), and WW = calf weaning weight (kg). In the very few studies where calf forage intake was recorded, there was little effect on ADG or weaning weight (Ansotegui et al., 1991), but level of milk intake affects voluntary forage intake by the calf (Broesder et al., 1990).

Predicting Intake from Expected or Desired Performance – Dry Matter Intake Required (**DMIR**). Anele et al. (2014) evaluated the feasibility of "back-calculating" DMI of growing-finishing cattle from observed or desired performance data. This approach has been applied in growing-finishing cattle for many years, typically being referred to as "programmed" or "prescription" feeding (Galyean, 1999). This programmed feeding method also has been applied to limit feeding of high-grain diets to gestating beef cows (Loerch, 1996; Gunter et al., 2000). Intake of DM using this approach is calculated by summing the NEm and net energy for gain (**NEg**) requirements of the animal divided by their respective dietary net-energy concentrations.

For this approach to be effective for either growing-finishing cattle or beef cows, assumptions are required, and critical pieces of information are needed. A key assumption is that cattle, at least over an extended period of time, will eat to meet energy needs for maintenance, growth, pregnancy, lactation, and so on. Thus, intake required to meet energy demands would match well with actual intake. As noted previously, intake seems to be highly variable in the short-term, but over the long term, this assumption seems reasonable. Information required includes BW, BCS, ADG, calf birth weights, milk production, and potentially climatic information that could be used to adjust for environmental effects. Perhaps the most critical piece of information is an accurate estimate of the dietary energy concentration. Ultimately, NE values are needed, but these are often determined from total digestible nutrients (**TDN**), digestible energy (**DE**), or metabolizable energy (**ME**) values (NASEM, 2016). For cattle in

confinement fed stored and milled concentrates, this information is readily obtainable and probably reasonably accurate. For cattle grazing forages, however, where selectivity of plant parts and plants species comes into play, as well as changes with advancing forage maturity, the reliability of energy values is open to question. This challenge is not unique to the DMIR approach because an energy value (or digestibility value as a proxy for energy) is also needed to predict DMI in the NASEM (2016) and Coleman et al. (2014) equations. Indeed, energy values for grazed forages are generally a "missing piece of the puzzle" when it comes to predicting DMI.

An Example of Applying the DMIR Approach. Developing a database to test the validity of using the DMIR approach in a manner similar to what Anele et al. (2014) did with growing-finishing cattle is technically impossible because grazed forage intake is measured indirectly. Thus, the "observed" intake is not directly measured and is subject to several potential sources of error. Nonetheless, it is possible to use data from confined livestock fed forage-based diets in which DMI, BW, and other production characteristics are measured by direct methods to evaluate how well the DMIR approach predicts observed DMI. Two studies from the literature were selected for this exercise that included growing heifers and beef cows. A brief description of each study follows. It should be noted as these examples were evaluated, however, that measurements of DMI with confined cattle are not made without error, and depending on the method, these errors could be substantial.

Buskirk et al. (1992) used 24 Angus cows to evaluate the relationships between energy intake, BW change, and BCS. Cows were allotted to 4 diets, including high-energy, maintenance-high, maintenance-low, and low-energy concentrations, and penned individually for measurement of feed intake. The DMI, along with changes in BW and BCS were recorded from d 12 to 200 postpartum, and milk production was estimated at 9 different times across the study by the weigh-suckle-weigh method. Trujillo et al. (2013) measured residual feed intake of heifers with potentially favorable allelic variant genes (referred to as the validation group) and a control group without the alleles. Measurements were made in confinement with a 60:40 concentrate:roughage diet and while the cattle were grazing on a high-quality oat pasture. Pasture intake was estimated using an n-alkane technique.

Results for the comparison of the observed DMI with DMI calculated using the DMIR method and the NASEM (2016) equations are shown in **Table 1**. Observed minus predicted values ranged from as little as 3.2 to 42.6% of the observed DMI for the DMIR method compared with 3.4 to 25.5% for the NASEM (2016) prediction equations. The DMIR method generally under-predicted DMI, which also was true for the NASEM (2016) equations. Of both studies evaluated, the predicted DMI values most closely matched the observed values for the Trujillo et al. (2013) study. This particular study was arguably the "simplest" of the studies, as the only energy requirements tabulated were for maintenance and gain. Determining requirements for pregnancy was challenging for studies involving pregnant females because of lack of clarity in terms of the number of days pregnant and the failure to report calf birth weights, which is needed to calculate the NEm requirement. Prediction errors with the DMIR method for lactating

beef cows (e.g., Buskirk et al., 1992) were particularly large, perhaps suggesting that refinement is needed in the NASEM (2016) maintenance requirements for lactating cows or that maintenance/lactation energy needs vary more with milk-producing ability of beef breeds than is currently accounted for in requirement equations. Finally, it is interesting that the DMIR method greatly under-predicted DMI with the high- and medium-grain diets in the Buskirk et al. (1992) study, but the predicted DMI was fairly close to the observed value for the low-energy diet.

Overall, the results for the DMIR method are somewhat disappointing. This might not be a particularly surprising result, however, as the net energy equations of NASEM (2016) are population-based equations, generally derived from empirical regression approaches that are not necessarily refined to the extent that they will fit all breed types and production/environmental settings. Biological variation in some of the components of these equations is large, and in many cases, the extent of such variation is not well-defined, particularly when it comes to grazing animals. For a definitive test of the DMIR approach with forage-fed and grazing cattle, a much larger and more robust database is needed.

Conclusions and Recommendations

Predicting intake by beef cattle raised in confinement and fed mixed or all-forage diets of consistent composition is not an easy task. Feed intake by beef cattle varies substantially from day to day. As a result, the “best” empirical equations with feedlot cattle, which are designed to predict DMI over extended periods of time, have prediction errors approximating 5% of the mean, with unexplained variation typically in the range of 25 to 50%. For grazing situations, where added variation results from selective grazing, sward characteristics, effects of advancing forage maturity, pre- and post-ingestive factors, social factors, climatic effects, landscape-related factors, and a host of other ill-defined effects, one would expect even greater prediction errors and more unexplained variation. Empirical equations can provide estimates of intake in grazing cattle, as can the DMIR method, which relies on the idea that energy demand drives long-term feed intake, thereby allowing energy requirements and diet energy concentrations to be used to predict DMI.

References

Anele, U. Y., E. M. Domby, and M. L. Galyean. 2014. Predicting dry matter intake by growing and finishing beef cattle: Evaluation of current methods and equation development. J. Anim. Sci. 92:2660-2667.

Ansotegui, R. P., K. M. Havstad, J. D. Wallace, and D. M. Halford. 1991. Effects of milk intake on forage intake and performance of suckling range calves. J. Anim. Sci. 69:899-904.

Broesder, J. T., M. B. Judkins, L. J. Krysl, S. A. Gunter, and R. K Barton. 1990. Thirty or sixty percent milk replacer reduction for calves: effects on alfalfa hay intake and digestibility, digestive kinetics and ruminal fermentation. J. Anim. Sci. 68:2974-2985.

Buskirk D. D., R. P. Lemenager, and L. A. Horstman. 1992. Estimation of net energy requirements (NE_m and NE_Δ) of lactating beef cows. J. Anim. Sci. 70:3867-3876.

Chadwick, M. A., P. V. Vercoe, I. H. Williams, and D. K. Revell. 2009. Programming sheep production on saltbrush: adaptions of offspring from ewes that consumed high amounts of salt during pregnancy and early lactation. Anim. Prod. Sci. 49:311-317.

Coleman, S. W., S. A. Gunter, J. E. Sprinkle, and J. P. S. Neel. 2014. Difficulties associated with predicting forage intake by grazing beef cows. J. Anim. Sci. 92:2775-2784.

Distel, R. A., J. J. Villalba, and H. E. Laborde. 1994. Effects of early experience on voluntary intake of low-quality roughage by sheep. J. Anim. Sci. 72:1191-1195.

FASS. 2010. Guide for the Care and Use of Agricultural Animals in Research and Teaching. 3rd ed. FASS, Inc., Champaign, IL.

Fisher, D. S. 2002. A review of a few key factors regulating voluntary feed intake in ruminants. Crop Sci. 42:1651-1655.

Forbes, J. M. 1986. The effects of sex hormones, pregnancy and lactation on digestion, metabolism, and voluntary food intake. Pages 420-435 in L. P. Milligan, W. L. Grovum, and A. Dobson, eds. Control of Digestion and Metabolism in Ruminants. Prentice Hall, Englewood Cliffs, NJ.

Forbes, J. M. 2003. The multifactorial nature of food intake control. J. Anim. Sci. 81:E139-E144.

Galyean, M. L. 1999. Review: Restricted and programmed feeding of beef cattle – Definitions, application, and research results. Prof. Anim. Sci. 15:1-6.

Ginane, C., M. Bonnet, R. Baumont, and D. K. Revell. 2015. Feeding behavior in ruminants: a consequence of interactions between a reward system and the regulation of metabolic homeostasis. Anim. Prod. Sci. 55:247-260.

Gunter, S. A., F. T. McCollum III, and R. L. Gillen. 1997. Forage intake by and site and extent of digestion in beef cattle grazing midgrass prairie rangeland and plains bluestem pasture throughout the summer. J. Anim. Sci. 75:490-501.

Gunter, S. A., P. A. Beck, J. S. Weyers, and K. A. Cassida. 2000. Programmed feeding for maintaining gestating beef cows in the Southeastern United States. Prof. Anim. Sci. 16:220-225.

Kennedy, P. M., R. J. Christopherson, and L. P. Milligan. 1986. Digestive responses to cold. Pages 285-306 in Control of Digestion and Metabolism in Ruminants, L. P. Milligan, W. L. Grovum, and A. Dobson, eds. Prentice-Hall, Englewood Cliffs, NJ.

Krysl, L. J., M. L. Galyean, M. B. Judkins, M. E. Branine, and R. E. Estell. 1987. Digestive physiology of steers grazing fertilized and nonfertilized blue grama rangeland. J. Range Manage. 40:493-501.

Kyriazakis, I., and J. D. Oldham. 1993. Diet selection in sheep: the ability of growing lambs to select a diet that meets their crude protein (nitrogen x 6.25) requirements. Br. J. Nutr. 69:617-629.

Leonardi, C., and L. E. Armentano. 2003. Effect of quantity, quality, and length of alfalfa hay on selective consumption by dairy cows. J. Dairy Sci. 86:557-564.

Loerch, S. C. 1996. Limit-feeding corn as an alternative to hay for gestating beef cows. J. Anim. Sci. 74:1211-1216.
NRC. 1985. Ruminant Nitrogen Usage. Natl. Acad. Press, Washington, DC.
NRC. 1987. Predicting Feed Intake of Food-Producing Animals. Natl. Acad. Press, Washington, DC.
NASEM. 2016. Nutrient Requirements of Beef Cattle, 8th ed. Natl. Acad. Sci. Eng. Med., Natl. Acad. Press, Washington, DC.
Odadi, W. O., M. K. Karachi, S. A. Abdulrazar, and T. P. Young. 2013. Protein supplementation reduces non-grass foraging by a primary grazers. Ecol. Appl. 23:455-463.
Provenza, F. D., P. Gregorini, and P. C. F. Carvalho. 2015a. Synthesis: foraging decisions link plants, herbivores and human beings. Anim. Prod. Sci. 55:411-425.
Provenza, F. D., M. Meuret, and P. Gregorini. 2015b. Our landscapes, our livestock, ourselves: Restoring broken linkages among plants, herbivores, and humans with diets that nourish and satiate. Appetite 95:500-519.
Scott, L. L., and F. D. Provenza. 2000. Lambs fed protein or energy imbalanced diets forage in locations and on foods that rectify imbalances. Appl. Anim. Behav. Sci. 68:293-305.
Senft, R. L., M. B. Coughenour, D. W. Baily, L. R. Rittenhouse, O. E. Sala, and D. M. Swift. 1987. Larger herbivore forage and ecological hierarchies. Bioscience 37:789-799.
Trujillo, A. I., A. Casal, F. Peñagaricano, M. Carriquiry, and P. Chilibroste. 2013. Association of SNP of neuropeptide Y, leptin, and IGF-1 genes with residual feed intake in confinement and under grazing condition in Angus cattle. J. Anim. Sci. 91:4235-4244.
Villalba, J. J., and F. D. Provenza. 1996. Preference for flavored wheat straw by lambs conditioned with intraruminal administrations of sodium propionate. J. Anim. Sci. 74:2362-2368.
Villalba, J. J., F. D. Provenza, F. Catanese, and R. A. Distal. 2015. Understanding and manipulating diet choice in grazing animals. Anim. Prod. Sci. 55:261-271.
Wiedmeier, R. W., J. J. Villalba, A. Summer, and F. D. Provenza. 2012. Eating a high fiber diet during pregnancy increases intake and digestibility of a high fiber by offspring in cattle. Anim. Feed Sci. Tech. 177:144-151.

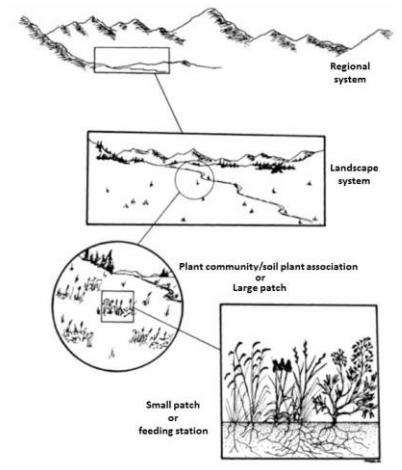


Figure 1. Different extents of spatial and temporal scales experienced by grazing cattle. Adapted from Senft et al. (1987), and published with permission from the Oxford University Press.

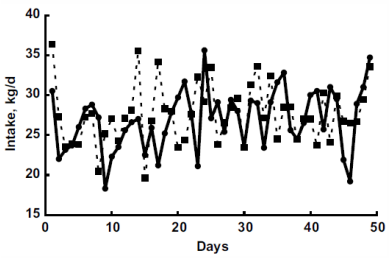


Figure 2. Intakes of grass silage by a beef animal. The solid line represents the observed data, whereas the dashed line represents random numbers with the same mean and standard deviation as the observed data. Adapted from Forbes (2003), and published with permission from the American Society of Animal Science.

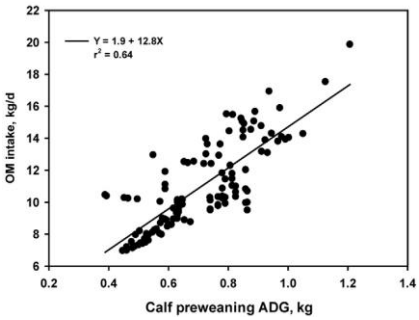


Figure 3. Relationship between OMI by cows and calf pre-weaning ADG. Adapted from Coleman et al. (2014), and published with permission from the American Society of Animal Science.

Table 1. Comparison of observed DMI and DMI predicted from observed performance (DMIR) and NRC (1996) equations

			kg/d				
Study/Group	Avg sBW, kg[1]	Diet NEm, Mcal/kg[2]	Obs DMI	DMIR[3]	Obs-Pred	NRC (1996)[4]	Obs-Pred
Buskirk et al. (1992)							
High	576	2.07	19.0	10.4	8.7	16.1	2.9
Maintenance-high	554	1.54	15.9	11.6	4.2	13.3	2.6
Maintenance-low	495	1.20	12.6	10.9	1.7	11.2	1.5
Low	471	1.12	9.8	10.4	-0.6	10.8	-1.1
Trujillo et al. (2013)							
Confinement-validation	214	1.61	6.7	6.3	0.3	5.5	1.2
Confinement-control	212	1.61	6.9	6.3	0.6	5.5	1.4
Grazing-validation	317	1.75	8.8	8.3	0.5	8.4	0.3
Grazing-control	327	1.75	10.9	8.4	2.5	8.6	2.3

[1]Average shrunk BW (0.96 × live BW was used when shrunk BW was not reported).
[2]Diet NEm concentration, DM basis.
[3]DMIR = DMI required to achieve observed performance based on NRC (1996) equations.
[4]NRC (1996) = equations for growing-finishing beef cattle and beef cows were used to predict DMI.

The Role of Rumen Microbiome on Feed Efficiency of Grazing Cattle

Leluo Guan[4], Fuyong Li, Anusha Bulumulla, Mi Zhou
Department of Agricultural Food and Nutritional Science
University of Alberta

Introduction

Feed efficiency of cattle directly affects the profitability, production efficiency, and is a determining factor for the sustainability of the beef industry, since feed cost could account for 50-70% of gross expenses (Cottle and Kahn, 2014). During cattle growth, approximately 75% of total dietary energy is used for maintenance (Arthur et al., 2001). Therefore, selection, breeding, and management of feed-efficient animals is a priority for the beef industry. Residual feed intake (**RFI**) is a measure of feed efficiency that is independent of growth and body weight. It has been identified as a selection tool for feed-efficient cattle based on this trait (Arthur et al., 2001; Basarab et al., 2003). It has been speculated that variation in RFI could be associated with many biological processes that are influenced by genetic and environmental factors; however, the molecular mechanisms underlying RFI are largely unknown.

In ruminant animals, the rumen plays a vital role in feed digestion and fermentation that produces short-chain fatty acids (**SCFAs**), which contribute up to 80% of the cattle's total energy requirements (Wolin, 1979). Diet can directly influence rumen function by altering the microbial population and fermentation activities (Bevans et al., 2005). A study by Durunna et al. (2011) revealed a re-ranking of RFI of individual cattle as they underwent a dietary change from a growing diet to a finishing diet in a feedlot production system. Therefore, we hypothesized that differences in the rumen microbiota could contribute to the observed variation of cattle feed efficiency. Our previous studies have revealed that particular microbes may be associated with cattle performance parameters including average daily gain, dry matter intake, feed conversion ratio, and RFI (Guan et al., 2008; Hernandez-Sanabria et al., 2010). The impact of these microbial populations on rumen function (fermentation measurements), RFI (Hernandez-Sanabria et al., 2012) and CH_4 emissions (Zhou et al., 2009; Zhou et al., 2010) has also been documented. Furthermore, particular microbial phylotypes in cattle arising from differing sires can influence rumen microbial metabolic processes and ultimately RFI (Hernandez-Sanabria et al., 2013). These suggest that rumen function (presence or absence of particular microbes) can be regulated by the interaction between 'gene (genotypes of the host)' and 'environment (diet, management)', which subsequently impact RFI ranking. Currently, there is no existing DNA marker for rumen function and the particular host mechanisms responsible for variation in the microbial populations, and their interactions with diet and impact on host feed efficiency are unknown.

[4] Contact information: Department of Agricultural, Food and Nutritional Science, University of Alberta. Edmonton, Alberta, Canada T6G2P5. E-mail: lguan@ualberta.ca.

Current Understanding of Rumen Microbiota

Ruminants are foregut fermenters characterized by their pre-gastric anaerobic fermentation in the rumen, where harbors variety of microbes including bacteria, archaea, protozoa, and fungi. The complex association of different microbes acts synergistically for the conversion of cellulosic feeds into volatile fatty acids (**VFAs**) and proteins that fulfill the nutrient requirement of animals (Frey et al., 2010). Rumen microbiology research has evolved in the last decade to understand their diversity, metabolic functions, and different interactions especially with the intervention of molecular biology techniques. To date, hundreds to thousands of microbial phylotypes have been identified from various rumen systems using the culture-independent molecular-based approaches (Brulc et al., 2009; Henderson et al., 2015). Such diverse microbial composition suggests that the rumen microbiome (collective genomes of rumen microbiota) contains 100 times more genes than the host animal (McSweeney and Mackie, 2012), providing genetic and metabolic capabilities to digest fibers and provide host animals with nutrients. The molecular microbial ecology studies have allowed the identification of uncultured and low abundant microbes, discovery of potential interactions among different microbial groups, and the quantitative exploration of this complex ecosystem which is co-evolved with their host. Many factors have been identified to affect rumen microbial diversity, density, and functions including diet, breed, age of the animal, physiological conditions and growth stages of the animals, season, geographic location, feed additives, feeding strategies, intensities, intake level, and animal health as well as medical treatment (antibiotic usage) (Weimer et al., 2000; Romero-Perez et al., 2011; Hernandez-Sanabria et al., 2012; McCann et al., 2014).
To date, numerous studies of analyzing rumen microbial communities using next generation sequencing estimated that the rumen microbiota contains up to approximately 7,000 bacterial species of which ~ 30% of them remain unidentified (McSweeney and Mackie, 2012). Among them,19 existing bacterial phyla have been identified with phyla of Firmicutes, Bacteroidetes, and Proteobacteria and genera of *Prevotella*, *Bacteroides,* and *Clostridia* dominating in most of the cattle rumens (Brulc et al., 2009; Cai et al., 2013). In addition, *Methanobrevibacter* (>60%), *Methanomicrobium* (~15%), and *Methanomassiliicoccales* (a group of uncultured rumen archaea previously referred to as rumen cluster C (RCC, ~16%) are the predominant genera in rumen archaeal community (St-Pierre and Wright, 2012; Borrel et al., 2014). Similar to other microbial groups, knowledge of protozoa has been significantly increased with the application of molecular techniques (Skillman et al., 2006). The most prevalent protozoans in the rumen can be classified under genus level, including *Epidinium*, *Entodinium*, *Diplodinium*, and *Holotrich* ciliates (Williams and Coleman 1992). Currently, more than 18 species of anaerobic rumen fungi have been described with the implementation of molecular biological techniques such as specific qPCR technique and high throughput sequencing technology (Denman et al., 2008). Rumen fungi have been classified into six genera; namely, the monocentric *Neocallimastix*, *Caecomyces, Piromyces,* and the polycentric *Anaeromyces*, *Orpinomyces,* and *Cyllamyces* (Ishaq, 2015). Last, it is noticeable that viruses, especially phages, are dense and diverse in the rumen. These were first identified in the 1960s but very few studies were done until the 1990s. Bacteriophages are abundant (10^7 to 10^9 particles per ml) in the rumen

ecosystem, and their population structure and symbiotic relationship are poorly understood (McSweeney and Mackie, 2012). Several studies have pointed out the influence of rumen viruses on other microbial population structure and density through cell lysis and possible lateral gene transfer (Hegarty and Klieve, 1999). Recent metagenomic analysis of bovine rumen virome identified 28,000 different viral genotypes belonging to several families (*Siphoviridae, Myoviridae, Podoviridae, Unclassified, Herpesviridae, Phycodnaviridae, Mimiviridae, Poxviridae, Baculoviridae, Iridoviridae, Polydnaviridae, Adenoviridae,* and *Bicaudaviridae*) (Berg Miller et al., 2012). They may play beneficial roles by balancing the bacterial populations, involving in lateral gene transfer, and adding novel enzymes to the rumen ecosystem and host animals, along with introducing detrimental effects such as reducing feed efficiency and transferring toxin genes (Gilbert and Klieve, 2015).

Advanced Methodologies to Study Rumen Microbiome

Rumen microbiome usually refers to the total genetic information of the rumen microbiota. There are two key questions when studying the rumen microbiome: Who are they and what are they doing in the rumen? By assesses the genomic information of the microbiota using metagenomics, it can help to identify the composition of the entire microbial community, to understand the symbiosis relationships between microbes and hosts, and to reveal the competition and communications within the microbiome (Handelsman, 2004). Brulc et al. (2009) firstly studied the metagenome of the rumen content collected from three beef steers, and have revealed fundamental variations in the glycoside hydrolases (GH) content of the steers fed on forages and legumes compared to that in the hindgut of the termite fed on wood. Hess et al. (2011) applied metagenomic analysis on the rumen microbiome of cows and have identified 27,755 putative carbohydrate-active genes, and expressed 90 candidate proteins among which 57% were active against cellulosic compounds of the feed. Besides, they have also assembled 15 unculturable microbial genomes, complementing the rumen microbial reference database. Microbial plasmids also encode essential functional genes. As reported by Kav et al. (2012), besides of the genes allowing the microbes to confer their host with advantages within the ecological riche, rumen microbial plasmidomes in cows also enriched in functions such as proximity to plasmid backbone functions and biosynthetic pathway function. Metagenomics help to discover the functional potentials within the rumen microbiome, but its actual activity has not been revealed. Thus, metatranscriptomics which study the active transcripts of microbial genes was then employed. Findley et al. (2011) isolated total RNA from cow rumen fluid and examined the transcripts of protozoan GHs, and identified four novel genes among which two (type 1-7.1 and type 2-8.6) were characterized in downstream biochemical assays. Metatranscriptomic analyses performed in cow rumen (Dai et al., 2012) have proved that the GHs produced by *Ruminococcus*, *Fibrobacter*, and *Prevotella* were the predominant degraders against plant cell wall polysaccharides (PCWP), with GH48 cellobiohydrolases and cellulosome-like structures contributed significant roles in efficient PCWP degradation. Getting the complete insight of rumen microbiome, it is also important to identify the microbial metabolites which can be utilized by the host or can influence rumen environment and host health. *Butyrivibrio proteoclasticus* B316^{T}, a

polysaccharide-degrading and butyrate-producing bacteria prevalent in the rumen, was reported to produce intracellular debranching enzymes, implicating a plausible model that this species is capable of conducting extracellular digestion of hemicellulose to oligosaccharides, followed by transporting the oligosaccharides to the cytoplasm for further digestion by intracellular enzymes (Dunne et al., 2015). Having these 'omics'-based approaches, it is possible to study the composition, activities, and functions of the rumen microbiome systematically.

Rumen Microbiome and Feed Efficiency

Beyond the previous predicted functions of microbes under specified experimental conditions, recent studies have added focus on the co-evolution (Ley et al., 2008; Hernandez-Sanabria et al., 2012) of gut microbes with the host and the possible interaction of animal's genotype with rumen microbes. With the developing knowledge about the rumen microbiota, it is feasible to explore the impacts of rumen microbiome to host performance by associating microbial measurements with host phenotypes. One of such application is to define the roles of rumen microbiota in affecting host feed efficiency. Hernandez-Sanabria et al. (2012) analyzed the rumen microbiome in beef cattle with varied RFI under growing and finishing diets, and found that the abundance of *Succinivibrio* sp. was associated with host dry matter intake and average daily gain in L-RFI (efficient) animals, *Robinsoniella* sp. abundance was associated with H-RFI (inefficient) animals, whereas the abundance of *Eubacterium* sp. differed between RFI groups when animals were fed with feedlot finishing diet. With a deeper coverage of sequences, Myer et al. (2015) reported that although Bacteroidetes and Firmicutes were the dominant phyla regardless of host feed efficiency differences, proportion of *Succiniclasticum*, *Lactobacillus*, *Ruminococcus*, and *Prevotella* differed among animal groups with varied feed intake and body weight gain. Jami et al. (2014) identified a tentative correlation between the relative abundance of bacteria order RF39 and host RFI (R=0.51) in dairy cows. Besides the findings on bacteria, both Zhou et al. (2009; 2010) and Carberry et al. (2014) reported that although the total methanogen population was similar, changes of particular archaeal genotype abundance may have attributed to the variation in host methane production and thereafter impacting host RFI.

Different Microbes are Associated With Beef Cattle Feed Efficiency

Recently, we applied a metatranscriptomic based approach to study the relationship between active rumen microbiome and feedlot cattle RFI. Both the bacterial community structure and the archaeal community structure were different ($P < 0.001$, using weighted UniFrac test) between H- and L-RFI groups. The relative abundance of three bacterial families including *Lachnospiraceae*, *Veillonellaceae*, p-2534-18B5, and one archaeal taxon *Methanomassiliicoccales* were different ($P < 0.05$) or tended to be different ($P < 0.10$) between H- and L-RFI steers (**Figure 1**). *Lachnospiraceae* has been reported to be associated with feed efficiency and fermentation traits in beef cattle in a previous study using DNA-based methods (Hernandez-Sanabria et al., 2010). Results of the current study showed that the H-RFI group possessed a larger relative abundance of *Lachnospiraceae* than the L-RFI group, further supporting its association with host RFI. In the rumen, *Veillonellaceae* can ferment lactate into acetate and

propionate (Dehority, 2003). The greater abundance of this phylotype in H-RFI animals suggested that lactate-reducing processes may be faster in H-RFI animals than in L-RFI animals. Future studies to measure the lactic acid in the rumen is needed to validate the role of this bacterial family in feed efficiency. *Methanomassiliicoccales* belongs to methylotrophic methanogens, which was the only methanogen known to use methylamines as the major energy and carbon sources (Poulsen et al., 2013). The higher relative abundance of *Methanomassiliicoccales* in L-RFI group indicated that more methylamines might be utilized during fermentation in L-RFI animals. Using 16S rRNA gene library sequencing on the same animals as the current study, Zhou et al. (2009) proposed that the varied methanogenesis substrate preferences may be one of the mechanisms leading to the variation in host CH_4 production between H- and L-RFI animals. The identified differential abundance of *Methanomassiliicocales* using transcriptomic analyses in current study further supported the linkage between methanogenic ecology and host feed efficiency, although the observed differences in arachael communities differed between the two studies. Additionally, *Methanomassiliicocales* is the only group encoding genes to synthesize pyrrolysine-containing proteins (Borrel et al., 2014), whose primary function is methylamine methyltransfer (Rother and Krzycki, 2010). The capability of utilizing a methyl-group from the methanogenesis substrates may lead to the variation of available energy and/or compounds to the host, and ultimately impacting host RFI. These results warrant further investigation to fully elucidate the relationship between available microbial metabolic substrates and host nutritional utilization pathways to better understand how microbial fermentation influences host feed efficiency.

Differential Microbial Functions Between H-RFI and L-RFI Animals

Functional analyses were also performed on the rumen microbiome of the 20 high and low efficiency steers. After quality control and removal of the ribosomal RNA (rRNA), the proportion of mRNA was 7.2 ± 0.5% (Mean ± SEM) of total reads among 20 samples. In total, 92,125,160 mRNA reads were subjected to the functional analysis. Between H-RFI and L-RFI groups, 1, 9 and 14 differential function features in the annotation sources of subsystems were detected at level 1, level 2, and level 3, respectively ($P < 0.05$). Among the listed functions that differed between H-RFI and L-RFI animals, it was noticeable that key metabolic pathways such as glycolysis and gluconeogenesis, purine and pyrimidine conversion, and pyruvate metabolism were more active in L-RFI steers, suggesting that the rumen microbiome in the L-RFI group were more active in digesting fibrous feed, and as such, supplied the host animals with more nutrients. In addition, the rumen microbiome in the L-RFI steers were more active in cell proliferation and survivability, and displayed higher tolerance to viral infection. These functional features may allow the rumen microbiome of L-RFI animals to better adapt to different environmental challenges, and as such improve rumen fermentation efficiency.

Implications for Grazing Systems

Although the current study was conducted on feedlot animals, the findings can be applied to animals in grazing systems. The grazing cattle production system is important

in North America for providing ecosystem goods and services such as forage, carbon storage, recreation as well as contributing to ecological diversity. Especially, for cow-calf production, grazing on summer pasture is a key period to produce beef at a lower cost, compared to feeding with grain. To date, the understanding of the rumen microbiota and its function in beef cattle has mainly focused on feedlot production systems, while the available information on grazing cattle is very limited due to the complexity and diversity of the production system (grazing rotation patterns, pasture diversity, intake monitoring and so on) and lack of access to allow the collection of phenotypic data and biological samples. Therefore, there is the need to perform more research in a collaborate manner to address the following fundamental questions: 1) what microorganisms are present in the rumen of cattle on pasture and what functional groups do they represent? 2) How does the nutritional and chemical composition of consumed forage from pasture affect the structure and function (microbial metabolites) of a microbial community? 3) Is the difference in rumen microbiota associated with cattle feed efficiency and predicted methane emission as is the case in feedlot production systems? This study aims to provide knowledge on the biological process (pasture digestion) of beef cattle production under grazing. With a more complete understanding of the microbial markers for better fiber digestibility and/or host feed efficiency, it is possible to design feed supplements for grazing animals to enhance their capability to utilize nutrients from pasture and/or improve feed efficiency thereby altering the rumen fermentation profiles. Furthermore, host genetics has been proposed to influence its symbiotic microbiota, and thus impact rumen fermentation processes. By defining the linkage between host genetics and microbial fermentation markers, it may be possible to provide novel tools from the microbial aspect to breed animals selectively, thus further enhancing animal performance and feed efficiency.

Conclusions

In conclusion, the exploration of the relationship between the activity of the rumen microbiome and host feed efficiency has revealed increased microbial metabolic functions in L-RFI steers, suggesting the important role of rumen microbiome in feed efficiency. Increased adaptability to negative environmental factors such as virus infection and higher cell survivability in L-RFI steers may enable their microbiome to adapt more quickly to adverse conditions, especially when animals are undergoing dietary challenges with poor quality diets. Given the dynamic nature of the rumen microbiome, future studies involving long-term monitoring of the microbial composition and functions are necessary to solidify its role in host RFI. Regardless, our findings provide new insights regarding the rumen microbiome in animals which differ in RFI ranking, providing the necessary knowledge to more fully understand rumen microbial fermentation, thereby enhancing nutrient utilization and improving animal feed efficiency through enhancing rumen fermentation.

References

Arthur, P. F., J. A. Archer, D. J. Johnston, R. M. Herd, E. C. Richardson, and P. F. Parnell. 2001. Genetic and phenotypic variance and covariance components for feed intake, feed efficiency, and other postweaning traits in Angus cattle. J, Animal Sci. 79:2805-2811.

Basarab, J. A., M. A. Price, J. L. Aalhus, E. K. Okine, W. M. Snelling, and K. L. Lyle. 2003. Residual feed intake and body composition in young growing cattle. Canadian J. Aimal Sci. 83:189-204.

Berg Miller, M. E., C. J. Yeoman, N. Chia, S. G. Tringe, F. E. Angly, R. A. Edwards, H. J. Flint, R. Lamed, E. A. Bayer, B. A. White. 2012. Phage-bacteria relationships and CRISPR elements revealed by a metagenomic survey of the rumen microbiome. Environ Microbiol. 14(1):207-27. doi: 10.1111/j.1462-2920.2011.02593.x.

Bevans, D. W., K. S. Beauchemin, K. S. Schwartzkopf-Genswein, J. J. McKinnon, and T. A. McAllister. 2005. Effect of rapid or gradual grain adaptation on subacute acidosis and feed intake by feedlot cattle. J. Animal Sci. 83:1116-1132.

Borrel, G., N. Parisot, H. M. Harris, E. Peyretaillade, N. Gaci, W. Tottey, O. Bardot, K. Raymann, S. Gribaldo, P. Peyret, P. W. O'Toole, and J. F. Brugère. 2014. Comparative genomics highlights the unique biology of *Methanomassiliicoccales*, a Thermoplasmatales-related seventh order of methanogenic archaea that encodes pyrrolysine. BMC genomics 15:679.

Brulc, J. M., D. A. Antonopoulos, M. E. B. Miller, M. K. Wilson, A. C. Yannarell, E. A. Dinsdale, P, M. Coutinho. 2009. Gene-centric metagenomics of the fiber-adherent bovine rumen microbiome reveals forage specific glycoside hydrolases. Proceedings of the National Academy of Sciences 106(6):1948-1953.

Carberry, C.A., D. A. Kenny, A. K. Kelly, and S. M. Waters. 2014. Quantitative analysis of ruminal methanogenic microbial populations in beef cattle divergent in phenotypic residual feed intake (RFI) offered contrasting diets. J. Animal Sci. and Biotechnology 5(1):1.

Dai, X., Y. Zhu, Y. Luo, L. Song, D., Liu, L. Liu, F. Chen, F., M. Wang, J. Li, X. Zeng, and Z. Dong. 2012. Metagenomic insights into the fibrolytic microbiome in yak rumen. PLoS One 7(7), e40430.

Cottle D., and L. Kahn. 2014. Beef cattle production and trade. CSIRO Publishing, Australia:221.

Dehority, B. A. 2003. Starch digesters, other less numerous species, and facultative anaerobes in the rumen Rumen Microbiology. p 243-264. Nottingham Univ. Press, UK.

Denman, S. E., M. J. Nicholson, J. L. Brookman, M. K. Theodorou, and C. S. McSweeney. 2008. Detection and monitoring of anaerobic rumen fungi using an ARISA method. Letters in Applied Microbiology 47(6):492-499.

Dunne, J. C., W. J. Kelly, S. C. Leahy, D. Li, J. J. Bond, L. Peng, G. T. Attwood, and T. W. Jordan. 2015. The Cytosolic Oligosaccharide-Degrading Proteome of Butyrivibrio Proteoclasticus. Proteomes 3(4):347-368.

Durunna, O. N., F. D. Mujibi, L. Goonewardene, E. K. Okine, J. A. Basarab, Z. Wang, and S. S. Moore. 2011. Feed efficiency differences and reranking in beef steers fed grower and finisher diets. J. Animal Sci 89:158-167.

Findley, S. D., M. R. Mormile, A. Sommer-Hurley, X. C. Zhang, P. Tipton, K. Arnett, J. H. Porter, M. Kerley, and G. Stacey. 2011. Activity-based metagenomic screening and biochemical characterization of bovine ruminal protozoan glycoside hydrolases. Applied and Environmental Microbiol. 77(22):8106-8113.

Frey, J. C., A. N. Pell, R. Berthiaume, H. Lapierre, S. Lee, J. K Ha, J. E. Mendell and E. R. Angert. 2010. Comparative studies of microbial populations in the rumen, duodenum, ileum and faeces of lactating dairy cows. J. Applied Microbiology 108(6):1982-1993.

Guan, L. L., J. D. Nkrumah, J. A. Basarab, and S. S. Moore. 2008. Linkage of microbial ecology to phenotype: correlation of rumen microbial ecology to cattle's feed efficiency. FEMS microbiology letters 288:85-91.

Handelsman, J. 2004. Metagenomics: application of genomics to uncultured microorganisms. Microbiology and molecular biology reviews, 68(4):669-685.

Hegarty, R.S., and A. V. Klieve. 1999. Opportunities for biological control of ruminal methanogenesis. Crop and Pasture Science, 50(8):1315-1320.

Henderson, G., F. Cox, S. Ganesh, A. Jonker, and W. Young, Collaborators, G.R.C. and P. H. Janssen. 2015. Rumen microbial community composition varies with diet and host, but a core microbiome is found across a wide geographical range. Scientific Reports 5:14567.

Hernandez-Sanabria, E., L. A. Goonewardene, Z. Wang, O. N. Durunna, S. S. Moore, and L. L. Guan. 2012. Impact of feed efficiency and diet on adaptive variations in the bacterial community in the rumen fluid of cattle. Applied and Environmental Microbiology 78:1203-1214.

Hernandez-Sanabria, E., L. A. Goonewardene, Z. Wang, M. Zhou, S. S. Moore, and L. L. Guan. 2013. Influence of sire breed on the interplay among rumen microbial populations inhabiting the rumen liquid of the progeny in beef cattle. PloS one 8.

Hernandez-Sanabria, E., L. L. Guan, L. A. Goonewardene, M. Li, D. F. Meiju, P. Stothard, S. S. Moore, and M. C. Leon-Quintero. 2010. Correlation of particular bacterial pcr-denaturing gradient gel electrophoresis patterns with bovine ruminal fermentation parameters and feed efficiency traits. Applied and Environmental Microbiology 76:6338-6350.

Hess, M., A. Sczyrba, R. Egan, T. W. Kim, H. Chokhawala, G. Schroth, S. Luo, D. S. Clark, F. Chen, T. Zhang, and R. I. Mackie. 2011. Metagenomic discovery of biomass-degrading genes and genomes from cow rumen. Science 331(6016): 463-467.

Ishaq, S. L., C. J. Kim, D. Reis, and A. D. G. Wright. 2015. Fibrolytic bacteria isolated from the rumen of North American moose (Alces alces) and their use as a probiotic in neonatal lambs. PloS ONE 10(12), e144804.

Jami, E., B. A. White, and I. Mizrahi. 2014. Potential role of the bovine rumen microbiome in modulating milk composition and feed efficiency. PLoS One 9(1), e85423

Kav, A. B., G. Sasson, E. Jami, A. Doron-Faigenboim, I. Benhar, and I. Mizrahi. 2012. Insights into the bovine rumen plasmidome. Proceedings of the National Academy of Sciences.

McCann, J. C., L. M. Wiley, T. D. Forbes, F. M. Rouquette, Jr., and L. O. Tedeschi. 2014. Relationship between the rumen microbiome and residual feed intake-efficiency of Brahman bulls stocked on bermudagrass pastures. PloS one, 9(3), e91864.

McSweeney, C., and Mackie, R. 2012. Commission on Genetic Resources for Food and Agriculture. Micro-organisms and ruminant digestion: State of knowledge, trends and future prospects. Background Study Paper (FAO), 61, 1-62.

Meyer, F., D. Paarmann, M. D'Souza, R. Olson, E. M. Glass, M. Kubal, T. Paczian, A. Rodriguez, R. Stevens, A. Wilke, J. Wilkening, and R. A. Edwards. 2008. The metagenomics RAST server - a public resource for the automatic phylogenetic and functional analysis of metagenomes. BMC bioinformatics 9:386.

Poulsen, M., C. Schwab, B. Borg Jensen, R. M. Engberg, A. Spang, N. Canibe, O. Højberg, G. Milinovich, L. Fragner, C. Schleper, W. Weckwerth, P. Lund, A. Schramm, and T. Urich. 2013. Methylotrophic methanogenic Thermoplasmata implicated in reduced methane emissions from bovine rumen. Nat communication: 4.

Romero-Pérez, G. A., K. H. Ominski, T. A. McAllister, and D. O. Krause. 2011. Effect of environmental factors and influence of rumen and hindgut biogeography on bacterial communities in steers. Appl. and Environ. Microbiology 77(1):258-268.

Rother, M., and J. A. Krzycki. 2010. Selenocysteine, pyrrolysine, and the unique energy metabolism of methanogenic archaea. Archaea 2010:453642.

Skillman, L. C., A. F. Toovey, A. J. Williams, and A. D. G. Wright. 2006. Development and validation of a real-time PCR method to quantify rumen protozoa and examination of variability between Entodinium populations in sheep offered a hay-based diet. Applied and environmental microbiology 72(1):200-206.

St-Pierre, B. and A. D. G. Wright. 2012. Molecular analysis of methanogenic archaea in the forestomach of the alpaca (Vicugna pacos). BMC microbiology 12(1), 1.

Williams, A.G. and G. S. Coleman. 2012. The rumen protozoa. Springer Science and Business Media.

Zhou, M., E. Hernandez-Sanabria, and L. L. Guan. 2009. Assessment of the microbial ecology of ruminal methanogens in cattle with different feed efficiencies. Applied and environmental microbiology 75:6524-6533.

Zhou, M., E. Hernandez-Sanabria, and L. L. Guan. 2010. Characterization of variation in rumen methanogenic communities under different dietary and host feed efficiency conditions, as determined by PCR-denaturing gradient gel electrophoresis analysis. Applied and environmental microbiology 76:3776-3786.

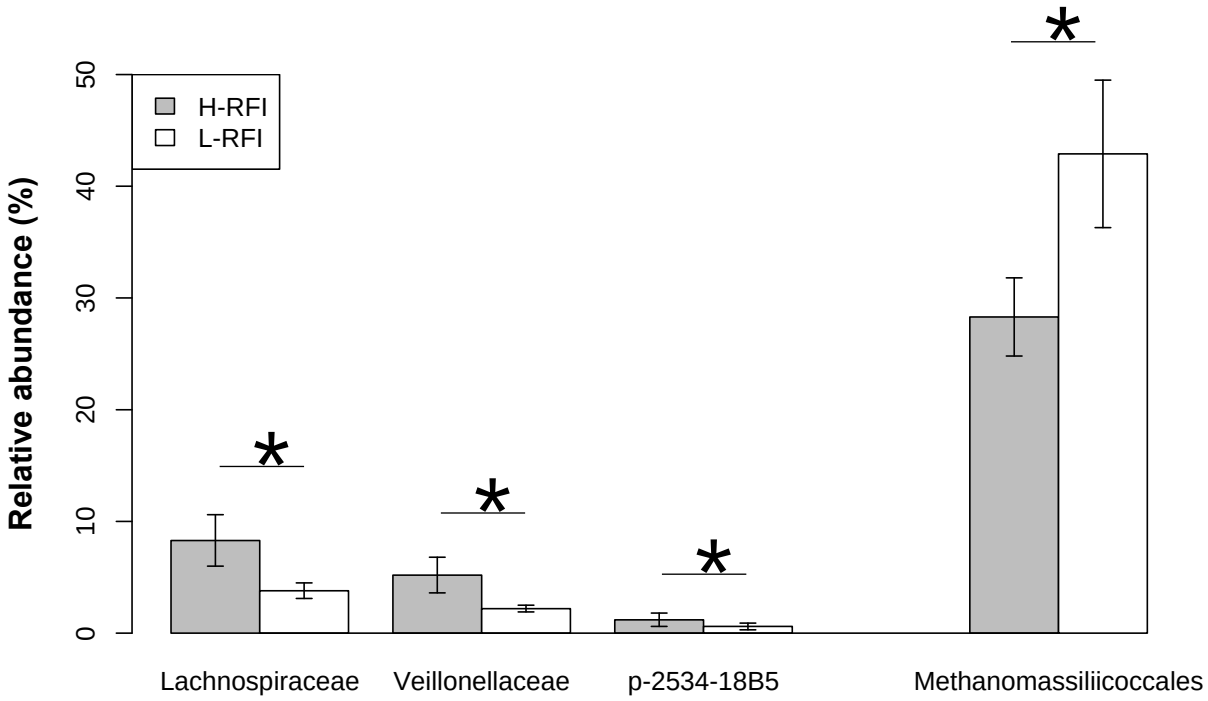


Figure 1. The relative abundance of active microbial taxa differed between H- and L-RFI groups. The relative abundance was calculated for bacterial and archaeal taxa separately. H- and L-RFI represent high and low residual feed intake, respectively. *P*-value was calculated using Metastats in Mothur and "*" represents *P*-value < 0.10.

Nutritional Mitigation of Greenhouse Gases

Diwakar Vyas
Department of Animal Sciences, University of Florida, Gainesville

Introduction

Methane (**CH_4**), carbon dioxide (**CO_2**), nitrous oxide (**N_2O**), and halocarbons are greenhouse gases (**GHG**) that are able to trap heat in the atmosphere by radiating less heat into the space and increase the effect of solar and thermal radiation on surface and atmospheric temperatures (Knapp et al., 2014). In 2014, total U.S. GHG emissions measured 6,870 million metric tons of CO_2 equivalents. Agricultural activities contributed about 9% of total GHG emissions (U.S. EPA, 2016). Enteric CH_4 generated during feed digestion accounts for most of livestock's direct impact on total GHG emissions representing about 28.6% of U.S. GHG emissions from agricultural activities in 2014 (U.S. EPA, 2016). Although CH_4 constitutes only about 10.6% of total emissions, it has greater impact because it has 28 times the global warming potential of CO_2 over a 100-yr timespan (Myhre et al., 2013). With an energy content of 55.22 MJ/kg (Brouwer, 1965), CH_4 represents a loss of dietary energy from the animal and typically accounts for about 6-12% of the total gross energy consumed by ruminants (Johnson and Johnson, 1995). Thus, CH_4 production by cattle is both an environmental concern and a potential loss in cattle efficiency. Reducing CH_4 losses is an environmentally sound practice with potential to improve production efficiency. Several comprehensive reviews have been published on strategies for CH_4 mitigation (Beauchemin et al., 2008; McAllister and Newbold, 2008, Hristov et al., 2013; Knapp et al., 2014). This paper will focus only on nutritional strategies to reduce enteric CH_4 emissions.

Nutritional strategies to reduce enteric CH_4 emissions

Dietary strategies to reduce CH_4 emissions were initially explored to increase energy efficiency. The first publication appearing in 1948 investigated the effects of dietary fat utilization and energy efficiency in sheep (Swift et al., 1948). However, the database on nutritional strategies to reduce CH_4 emissions has grown exponentially in last two decades after initial publication on the impact of ruminants on GHG emissions (Johnson and Johnson, 1995). Recently, databases generated for quantification of mitigation strategies for enteric CH_4 emission has shown that dietary manipulation by increasing or substituting concentrates in the diet or lipid supplementation has received greater attention to reduce enteric CH_4 emissions because of their effects on energy use efficiency and production (Veneman et al., 2016). Similarly, the efficacy of improved forage quality has been well explored. However, in the last decade research has focused on inhibiting methanogens and targeting rumen fermentation by use of secondary plant metabolites (tannins or saponins), electron acceptors (nitrate), or feed additives (3-nitrooxypropanol) to reduce CH_4 production. This paper aims to summarize

[1] Contact: 2250 Shealy Drive, Gainesville, FL 32611, Telephone: (352) 294-1079, E-Mail: diwakarvyas@ufl.edu

nutritional strategies for reducing enteric CH_4 emissions relevant to the ruminant production systems and will be primarily focused on highlighting three strategies considered most effective in reducing enteric CH_4 emissions; namely, lipid supplementation, dietary nitrate, and 3-nitrooxypropanol.

Lipid supplementation

Dietary fat is among the most promising strategies for reducing enteric CH_4 emissions (Beauchemin et al., 2008; Grainger and Beauchemin, 2011; Patra, 2013). Dietary fat reduces CH_4 emissions by decreasing organic matter fermentation in the rumen along with reducing the protozoal numbers (Martin et al., 2016) and activity of methanogens (Popova et al., 2011; Guyader et al., 2015). Lipids with greater proportions of polyunsaturated fatty acids (**PUFA**) may help in reducing methanogenesis via channeling hydrogen towards ruminal biohydrogenation of unsaturated fatty acids; however, based on stoichiometric (Czerkawski, 1986) and modeling approaches (Mills et al., 2001), only 1-2% of metabolic hydrogen in the rumen is used for this purpose.

The efficacy of adding dietary lipids to reduce CH_4 emissions is affected by various factors including fat source, fatty acid profile, form in which fat is administered (i.e. either as refined oil or as full-fat oilseeds), level of supplementation, and the type of diet. Grainger and Beauchemin (2008) observed a linear decline in CH_4 production with increasing level of total fat ranging from 1 to 13.1% of dietary DM from 27 studies (**Figure 1**). Similar results were observed when total dietary fat concentration was restricted to < 8% of dietary DM. In another meta-analysis study, Patra (2013) indicated that enteric CH_4 emissions (g/kg of DM or g/kg of milk) declined linearly with increasing dietary lipid concentration when total fat concentration was restricted to < 5% in diets. Based on the results observed from previous meta-analysis studies, it was interpreted that with each percentage unit increase in total fat concentration, CH_4 emissions will be reduced by 0.66 g/kg of DMI (Patra, 2013), 1 g/kg of DMI (Grainger and Beauchemin, 2011), or 0.79 g/kg of DMI (Moate et al., 2011).

Fatty acid composition of dietary fat seems to have an inconsistent effect on CH_4 yield. While Grainger and Beauchemin (2011) observed no relationship between fatty acid composition and CH_4 yield, Martin et al. (2010) observed greater CH_4 reduction with lauric acid (**C12:0**) and myristic acid (**C14:0**). Similarly, Patra (2013) reported C12:0 and PUFA (**C18:3**) as potent inhibitors of methanogenesis while fatty acids C16:0, C18:0, and C18:2 were not effective at reducing CH_4 emissions. Previous studies have proposed that medium chain fatty acids mitigate CH_4 emissions by reducing the abundance and metabolic activity of methanogens (Lillis et al., 2011; Patra and Yu, 2013) while the effects unsaturated fatty acids might be mediated via reducing abundance of methanogens and channeling hydrogen during the biohydrogenation process.

While the effects of dietary lipids on methanogenesis has been well studied, most of the previous studies were short-term and we are still lacking enough literature on the persistence of the anti-methanogenic potential of lipid supplementation (Hristov et al.,

2013). Woodward et al. (2006) reported short-term efficacy of vegetable and fish oil in reducing CH_4 emissions in pasture-fed dairy cows; however, the effects disappeared after 11 wk of feeding lipids. On the contrary, the effects of extruded linseed in reducing CH_4 emissions persisted for one year in dairy cows fed diets based on grazed pasture or grass silage (Martin et al., 2011). Similarly, Grainger and Beauchemin (2011) analyzed 6 long-term studies and reported greater persistence of reduced CH_4 emissions with dietary fat; however, results were inconsistent.

The mitigation strategies using dietary fats should carefully consider its negative impact on DMI, milk yield, and milk fat and protein concentration. Patra (2013) observed increased milk yield in response to fat supplementation; however, while milk yield increased initially, plateau was reached between 3.9-6% total dietary fat concentration and milk yield decreased thereafter. Similarly, DMI levels decreased when dietary fat concentration was > 4.2%. In addition, DM and neutral detergent fiber (**NDF**) digestibility was linearly reduced with increasing fat concentration (Patra, 2013). Previous reviews have also observed negative effects on intake levels with lipid supplementation (Chilliard, 1993; Allen, 2000). While rumen inert fat sources did not affect DMI, oil sources (vegetable oils, medium chain fatty acids) significantly reduced DMI (Knapp et al., 2014). Some lipid sources like vegetable oil, containing unsaturated fatty acids or coconut oil containing medium chain fatty acids might have greater efficacy in reducing CH_4 emissions; however, it might largely be achieved by reduced intake levels, thereby reducing milk yield in the long-term. Lipids causing this kind of production effect cannot be recommended as mitigation agents (Hristov et al., 2013).

Nitrate supplementation.

The CH_4 mitigation potential of supplemental nitrate has received considerable attention recently as nitrate can act as an alternative hydrogen sink in the rumen that competes with CH_4 formation (Lee and Beauchemin, 2014). In the rumen, nitrate is first reduced to nitrite, and is then further reduced to ammonia. Nitrate reduction is considered a thermodynamically more favorable pathway than the reduction of CO_2 to CH_4 and therefore suppresses CH_4 production (Lee and Beauchemin, 2014). Dietary nitrate as a feed additive to mitigate enteric CH_4 emissions is considered an effective strategy based on its consistent and persistent efficacy between studies (Lee et al., 2015). Recently, a meta-analysis from 8 studies including data from sheep, beef cattle, and dairy cattle showed a linear decline in CH_4 production with increasing intake of dietary nitrate per kg of BW (**Figure 2**; Lee and Beauchemin, 2014). Similarly, several studies have reported CH_4 mitigation in the range of 16-25% in CH_4 yield (g/kg of DMI) at nitrate inclusion levels of 2.1% of DMI (van Zijderveld et al., 2011; Lund et al., 2014; Lee et al., 2015; Klop et al., 2016; Olijhoek et al., 2016) in dairy cattle. In addition, long-term persistence of CH_4 mitigation has also been reported with dietary nitrate (Li et al., 2012; El-Zaiat et al., 2014) further confirming the usefulness of feeding nitrate as a potential strategy to mitigate enteric CH_4 emissions from ruminants. The combination of nitrate with other mitigation strategies such as sulfate (van Zijderveld et al., 2010) and linseed oil (Guyader et al., 2015) has been shown to be additive in terms of reducing CH_4 emissions. However, the barrier to the use of nitrate in practical feeding conditions is its potential toxicity. As mentioned earlier, dietary nitrate introduced into the rumen is

reduced to nitrite and ammonia. However, depending on the rate of nitrate reduction, nitrate and nitrite can accumulate in ruminal fluid and absorbed via the rumen wall. While nitrate that appears in blood is not toxic, nitrite binds to red blood cells, gets oxidized to nitrate and changes the ferrous (Fe^{2+}) form of haemoglobin to the ferric (Fe^{3+}) form (methemoglobin) resulting in reduced oxygen carrying capacity of blood causing tissue hypoxia and death (Bruning-Fann and Kaneene, 1993; Leng, 2008). Various factors affect potential toxicity of nitrate in ruminants including the levels and consumption rate of dietary nitrate, along with nitrate and nitrite reducing capacity in the rumen (Lee et al., 2015). The strategy to lower nitrate toxicity includes acclimation by gradual increase in supplemental nitrate thereby increasing the population of ruminal microbes that are able to reduce nitrate and nitrite to ammonia. Shi et al. (2012) confirmed greater nitrate reduction in ruminal fluid from sheep acclimated to nitrate.

From a nutritional perspective, nitrate could potentially replace urea as a non-protein nitrogen (**NPN**) source for microbial protein synthesis as shown by comparable effects on feed intake and production levels in ruminants (Li et al., 2012; El-Zaiat et al., 2014) ensuring that levels of dietary nitrate are below the levels causing potential toxicity and that a proper acclimation period is used. In addition, previous studies have reported either no effects (Nolan et al., 2010; Li et al., 2012) or greater (Lee et al., 2015) total-tract DM digestibility in response to supplemental nitrate. Hence, with no effects on DM digestibility and significant CH_4 mitigation, supplemental nitrate has the potential to increase energy efficiency and productivity in ruminants. However, previous studies conducted over short-term (Lee et al., 2015) and long-term (Li et al., 2012) periods have observed no improvement in production by feeding nitrate. Similarly, Lee and Beauchemin (2014) observed no responses of live weight gain to feeding nitrate in ruminants. Lack of effects on production might be attributed to inefficient energy utilization of hydrogen when used to reduce nitrate to ammonia compared to when used for methanogenesis because 44% of free energy is lost during nitrate reduction compared to a 6% energy loss during CH_4 formation (van Zijderveld, 2011).

3-nitrooxypropanol.

3-Nitrooxypropanol (**3-NOP**) is a novel strategy to reduce CH_4 production by inhibiting methyl-coenzyme M reductase (**MCR**), which catalyzes the biosynthesis of CH_4 (Duin et al., 2016). It has been suggested that 3-NOP, at micromolar concentration, inactivates MCR by oxidation of its active site (Ni^{+1}) required for the CH_4-forming step in rumen fermentation. Also, inhibitory effects of 3-NOP were demonstrated against methanogenic archaea without inducing any effects on growth of non-methanogenic bacteria in the rumen (Duin et al., 2016).

The first in vitro study to investigate the efficacy of 3-NOP reported an 86-96% reduction in CH_4 production without affecting the concentration of volatile fatty acids (**VFA**). This was followed by in vivo experiments with sheep which demonstrated a 26% reduction in CH_4 yield (g/kg of DMI) with 3-NOP provided at 100 g/d (Martinez-Fernandez et al., 2014). While no negative effects were observed on DMI or live weight gain, CH_4 reduction was accompanied by a reduced acetate-to-propionate ratio (Martinez-Fernandez et al., 2014). Similarly, Reynolds et al. (2014) reported a 4.4 and a

6.7% reduction in CH_4 production with 3-NOP supplemented at 0.5 and 2.5 g/d without affecting DMI, digestibility, or milk yield. However, CH_4 mitigation was accompanied by a reduction in total methanogens, VFA, and molar proportion of acetate while propionate proportion was increased with a higher dose of 3-NOP. Haisan et al. (2014) observed a 60% reduction in CH_4 production with 3-NOP provided at 2.5 g/d. The greater efficacy in reducing CH_4 emissions in this study was attributed to the mode of providing NOP to the cows (mixed with the feed) compared to ruminal dosing in the earlier study (Reynolds et al., 2014). Recently, Hristov et al. (2013) observed an average 30% reduction in CH_4 production when NOP was added to the diet of lactating dairy cows at 40, 60, and 80 mg of NOP/kg of DM over 12 weeks. No adaption to 3-NOP was reported. Both previous studies (Haisan et al., 2013; Hristov et al., 2015) observed increased BW gain suggesting partial redirection of energy from CH_4 to tissue deposition for cows receiving 3-NOP. Likewise, in beef cattle, Romero-Perez et al. (2014) investigated 3 doses of 3-NOP equivalent to 0.75, 2.25 and 4.50 mg/kg of DM and observed a 33% CH_4 reduction at the highest level of supplementation along with a linear reduction in the acetate-to-propionate ratio. No effects were observed on diet digestibility. In another study, Romero-Perez et al. (2015) reported a sustained decrease of methanogenesis for 112 d. Similarly, sustained reduction of enteric CH_4 emissions was demonstrated in beef cattle fed backgrounding and finishing diets for 105 d each. While the extent of CH_4 mitigation with a backgrounding diet (**Figure 3**) was 29% (g/kg of DMI), an 84% reduction in CH_4 emissions was observed with 3-NOP supplemented at 200 mg/kg of DM with high-grain diets (Vyas et al., 2016a). Furthermore, gain-to-feed ratio tended to increase in animals fed high-forage diets supplemented with 3-NOP (**Figure 4**) and it can be speculated that moderate reductions in CH_4 emissions (approximately 30%) appears to be associated with improved performance and energy efficiency, perhaps because the changes in the rumen ecosystem are not drastic (Vyas et al., 2016a). While strong reductions (approximately 80%) in CH_4 emissions might spare energy, negative effects on the rumen ecosystem leading to reduced utilization of the spared energy cannot be overlooked. Vyas et al. (2016b) reported that the optimal dose of 3-NOP supplementation in beef cattle fed high-forage and high-grain diets ranges from 100-200 mg/kg of DM for reducing CH_4 emissions without inducing any negative effects on production parameters.

Hence, based on results from previous studies, dietary supplementation of 3-NOP has consistently reduced enteric CH_4 emission. Moreover, no adaptation to 3-NOP was observed when supplemented over the long-term. Additionally, no negative effects were observed on nutrient digestibility and animal performance and no risk in terms of food safety have been reported to date. However, this product in not available for commercial use as toxicology studies are still being carried out to support registration as a feed additive in the U.S.

Conclusions

Nutritional manipulation is an effective strategy to reduce enteric CH_4 emissions and its impact would be achieved by approaches that would be feasible under practical feeding conditions. Farmers would tend to choose options for CH_4 mitigation that are simple, cost-effective, and without compromising feed efficiency and farm profitability.

Dietary strategies discussed in this paper (lipids, nitrate, and 3-NOP) are promising in persistently reducing CH_4 emissions. While guidelines for addition of fat to TMR diets have been developed to maximize milk production, future studies are still required to establish nitrate and 3-NOP as feed additives.

The demand for animal source food to feed an increasing world population will require more animals, and so total global CH_4 emissions will increase. However, strategies to mitigate CH_4 emissions should focus on reducing the amount of emissions/kg of livestock product. Development of mitigation strategies to reduce CH_4 emissions, while not lowering animal production, is critical to achieving this goal.

References

Allen, M. S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J. Dairy Sci. 83:1598–1624.

Beauchemin, K. A., M. Kreuzer, F. O'Mara, and T. A. McAllister. 2008. Nutritional management for enteric methane abatement: a review. Aust. J. Exp. Agri. 48:21-27.

Brouwer, E. 1965. Report of subcommittee on constants and factors. Proc. 3rd EAAP Symposium on energy metabolism pp. 441-443. Troon, Publ. 11, Academic Press, London.

Brunning-Fann, J. S., and J. B. Kaneene. 1993. The effects of nitrate, nitrite, and N-nitroso compounds on animal health. Vet. Hum. Toxicol. 35:237-253.

Chilliard, Y. 1993. Dietary fat and adipose tissue metabolism in ruminants, pigs, and rodents: A review. J. Dairy Sci. 76:3897–3931.

Czerkawski, J. W. 1986. An introduction to rumen studies. Pergamon Press, Oxford, UK.

Duin, E. C., T. Wagner, S. Shima, D. Prakash, B. Cronin, D. R. Yanez-Ruiz, S. Duval, R. Rumbeli, R. T. Stemmler, R. K. Thauer, and M. Kindermann. 2016. Mode of action uncovered for the specific reduction of methane emissions from ruminants by the small molecule 3-nitrooxypropanol. Proc. Nat. Acad. Sci. 113:6172-6177.

Duval, S., and M. Kindermann. 2012. Use of nitooxy organic molecules in feed for reducing enteric methane emissions in ruminants, and/or to improve ruminant performance. International Patent Application WO 84629: A1.

El-Zaiat, H. M., R. C. Araujo, Y. A. Soltan, A. S. Morsy, H. Louvandini, A. V. Pires, H. O. Patino, P. S. Correa, and A. L. Abdalla. 2014. Encapsulated nitrate and cashew nut shell liquid on blood and rumen constituents, methane emission, and growth performance of lambs. J. Anim. Sci. 92:2214–2224.

Grainger, C., and K. A. Beauchemin. 2011. Can enteric methane emissions from ruminants be lowered without lowering their production? Anim. Feed Sci. Tech. 166-167:308-320.

Guyader, J. M., M. Eugene, B. Meunier, M. Doreau, D. P. Morgavi, M. Silberberg, Y. Rochette, C. Gerard, C. Loncke, and C. Martin. 2015. Additive mitigating effect between dietary linseed oil and nitrate fed to cattle. J. Anim. Sci. 93:3564-3577.

Haisan, J., Y. Sun, L. L. Guan, K. A. Beauchemin, A. Iwaasa, S. Duval, D. R. Barreda, and M. Oba. 2014. The effects of feeding 3-nitrooxypropanol on methane

emissions and productivity of Holstein cows in mid lactation. J. Dairy Sci. 97:3110-3119.
Hristov, A. N., O. Joonpyo, G. Fabio, Y. W. Frederick, M. T. Harper, H. L. Weeks, A. F. Branco, P. J. Moate, M. H. Deighton, S. R. O. Williams, M. Kindermann, and S. Duval. 2015. An inhibitor persistently decreased enteric methane emission from dairy cows with no negative effect on milk production. Proc. Natl. Acad. Sci. 112:10663-10668.
Johnson, K. A., and D. E. Johnson. 1995. Methane emissions from cattle. J. Anim. Sci. 73:2483–2492.
Klop, G., B. Hatew, A. Bannink, and J. Dijkstra. 2016. Feeding nitrate and docosahexaenoic acid affects enteric methane production and milk fatty acid composition in lactating dairy cows. J. Dairy Sci. 99:1161–1172.
Knapp, J. R., G. L. Laur, P. A. Vadas, W. P. Weiss, and J. M. Tricarico. 2016. Enteric methane in dairy cattle production: Quantifying the opportunities and impact of reducing emissions. J. Dairy Sci. 97:3231-3261.
Lee, C., and K. A. Beauchemin. 2014. A review of feeding supplementary nitrate to ruminant animals: Nitrate toxicity, methane emissions, and production performance. Can. J. Anim. Sci. 94:557–570.
Lee, C., R. C. Araujo, K. M. Koenig, and K. A. Beauchemin. 2015. Effects of feed consumption rate of beef cattle offered a diet supplemented with nitrate adlibitum or restrictively on potential toxicity of nitrate. J. Anim. Sci. 93:4956-4966.
Leng, R. A. 2008. The potential of feeding nitrate to reduce enteric methane production in ruminants. Report to Department of Climate Change, Commonwealth Government, Canberra. [Online] Available: http://www.penambulbooks.com/Downloads/Leng-Final Modified 17-9-2008.pdf [2010 Jul. 17].
Li, L., J. Davis, J. Nolan, and R. Hegarty. 2012. An initial investigation on rumen fermentation pattern and methane emission of sheep offered diets containing urea or nitrate as the nitrogen source. Anim. Prod. Sci. 52:653–658.
Lillis, L., B. Boots, D. A. Kenny, K. Petrie, T. M. Boland, N. Clipson, and E. M. Doyle. 2011. The effect of dietary concentrate and soya oil inclusion on microbial diversity in the rumen of cattle. J. Appl .Microbiol. 111:1426–1435.
Lopes, J. C., M. T. Harper, F. Giallongo, J. Oh, L. Smith, A. M. Ortega-Perez, S. A. Harper, A. Melgar, D. M. Kniffen, R. A. Fabin, and A. N. Hristov. 2017. Effect of high-oleic-acid soybeans on production performance, milk fatty acid composition, and enteric methane emission in dairy cows. J. Dairy Sci. (In press) http://dx.doi.org.lp.hscl.ufl.edu/10.3168/jds.2016-11911
Lund, P., R. Dahl, H. J. Yang, A. L. F. Hellwing, B. B. Cao, and M. R. Weisbjerg. 2014. The acute effect of addition of nitrate on in vitro and in vivo methane emission in dairy cows. Anim. Prod. Sci. 54:1432–1435
Martin, C., D. P. Morgavi, and D. Moreau. 2010. Methane mitigation in ruminants: from microbe to the farm scale. Animal 4:351–365.
Martin, C., A. Ferlay, P. Mosoni, Y. Rochette, Y. Chilliard, and M. Doreau. 2016. Increasing linseed supply in dairy cow diets based on hay or corn silage: Effect on enteric methane emission, rumen microbial fermentation, and digestion. J. Dairy Sci. 99:3445-3456.

Martinez-Fernandez, G., L. Abecia, A. Arco, G. Cantalapiedra-Hijar, A. I. Martín-García, E. Molina-Alcaide, M. Kindermann, S. Duval, and D. R. Yáñez-Ruiz. 2014. Effects of ethyl-3-nitrooxy propionate and 3-nitrooxypropanol on ruminal fermentation, microbial abundance, and methane emissions in sheep. J. Dairy Sci. 97:3790-3799.

McAllister, T.A., and C. J. Newbold. 2008. Redirecting rumen fermentation to reduce methanogenesis. Aust. J. Exp. Agri. 48:7-13.

Mills, J. A. N., J. Dijkstra, A. Bannink, S. B. Cammell, E. Kebreab, and J. France. 2001. A mechanistic model of whole-tract digestion and methanogenesis in the lactating dairy cow: Model development, evaluation and application. J. Anim. Sci. 79:1584-1597.

Myhre, G., D. Shindell, F. M. Bréon, W. Collins, J. Fuglestvedt, J. Huang, D. Koch, J. F. Lamarque, D. Lee, B. Mendoza, T. Nakajima, A. Robock, G. Stephens, T. Takemura, and H. Zhang. 2013. Anthropogenic and Natural Radiative Forcing. Pages 8SM1– 8SM44 in Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. T. F. Stocker, D. Qin, G. K. Plattner, M. S. Tignor, K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex, and P. M. Midgley, ed. Cambridge University Press, Cambridge, United Kingdom and New York, NY.

Nolan, J. V., R. S. Hegarty, J. Hegarty, I. R. Godwin, and R. Woodgate. 2010. Effects of dietary nitrate on fermentation, methane production, and digesta kinetics in sheep. Anim. Prod. Sci. 50:801–806.

Patra, A. K. 2013. The effect of dietary fats on methane emissions, and its other effects on digestibility, rumen fermentation and lactation performance in cattle: A meta-analysis. Livest. Sci. 155:244-254.

Patra, A. K. and Z. Yu. 2013. Effects of coconut and fish oils on methane production, fermentation, abundance and diversity of rumen microbial populations in vitro. J. Dairy Sci. 96:1782-1792.

Popova, M. C., C. Martin, M. Eugene, M. M. Mialon, M. Doreau, and D. P. Morgavi. 2011. Effect of fiber- and starch- rich finishing diets on methanogenic archaea diversity and activity in the rumen of feedlot bulls. Anim. Feed Sci. Technol. 1166:113-121.

Romero-Perez, A. E. K. Okine, S. M. McGinn, L. L. Guan, M. Oba, S. M. Duval, M. Kindermann, and K. A. Beauchemin. 2014. The potential of 3-nitrooxypropanol to lower enteric methane emissions from beef cattle. J. Anim. Sci. 92:4682-4693.

Romero-Perez, A. E. K. Okine, S. M. McGinn, L. L. Guan, M. Oba, S. M. Duval, M. Kindermann, and K. A. Beauchemin. 2015. Sustained reduction in methane production from long-term addition of 3-nitrooxypropanol to a beef cattle diet. J. Anim. Sci. 93:1780-1791.

Shi, C., Q. Meng, X. Hou, L. Ren, and Z. Zhou. 2012. Response of ruminal fermentation, methane production and dry matter digestibility to microbial source and nitrate addition level in an in vitro incubation with rumen microbes obtained from wethers. J. Anim. Vet. Adv. 11:3334-3341.

Swift, R. W., J. W. Bratzler, W. H. James, A. D. Tillman, and D. C. Meek. 1948. The effect of dietary fat on utilization of the energy and protein of rations by sheep. J. Anim. Sci. 7:475–485.
van Zijderveld, S. M., W. J. J. Gerrits, J. A. Apajalahti, J. R. Newbold, J. Dijkstra, R. A. Leng, and H. B. Perdok. 2010. Nitrate and sulfate: Effective alternative hydrogen sinks for mitigation of ruminal methane production in sheep. J. Dairy Sci. 93:5856–5866
van Zijderveld, S. M. 2011. Dietary strategies to reduce methane emissions from ruminants. Ph.D. thesis. Wageningen University, the Netherlands.
van Zijderveld, S. M., W. J. J. Gerrits, J. Dijkstra, J. R. Newbold, R. B. A. Hulshof, and H. B. Perdok. 2011. Persistency of methane mitigation by dietary nitrate supplementation in dairy cows. J. Dairy Sci. 94:4028-4038.
Veneman, J. B., E. R. Saetnan, A. J. Clare, and C. J. Newbold. 2016. MitiGate; an online meta-analysis database for quantification of mitigation strategies for enteric methane emissions. Sci. Total Environ. 572:1166-1174.
Woodward, S. L., G. C. Waghorn, and N. A. Thomson. 2006. Supplementing dairy cows with oils to improve performance and reduce methane- Does it work? Proc. N. Z. Soc. Anim. Prod. 66:176-181.
U.S. EPA (Environmental Protection Agency). 2016. Inventory of U.S. Greenhouse Gas Emissions and Sinks: 1990–2014. Washington, DC: U.S. EPA. https://www.epa.gov/sites/production/files/2016-04/documents/us-ghg-inventory-2016-main-text.pdf
Vyas, D., S. M. McGinn, S. M. Duval, M. Kindermann, and K. A. Beauchemin. 2016a. Effects of sustained reduction of enteric methane emissions with dietary supplementation of 3-nitrooxypropanol on growth performance of growing and finishing beef cattle. J. Anim. Sci. 94:2024-2034.
Vyas, D., S. M. McGinn, S. M. Duval, M. K. Kindermann, and K. A. Beauchemin. 2016b. Optimal dose of 3-nitrooxypropanol for decreasing enteric methane emissions from beef cattle fed high-forage and high-grain diets. Anim. Prod. Sci. http://dx.doi.org/10.1071/AN15705

Figure 1. Linear and curvilinear relationships between dietary fat concentration and CH_4 yield. Linear equation: Y = 24.65 (±0.890) - 0.103 (±0.0109)X; curvilinear equation Y = 26.50 (±1.270)− 0.187 (±0.0430)X + 0.0007 (±0.00037)X^2 (Adapted from Grainger and Beauchemin,2011).

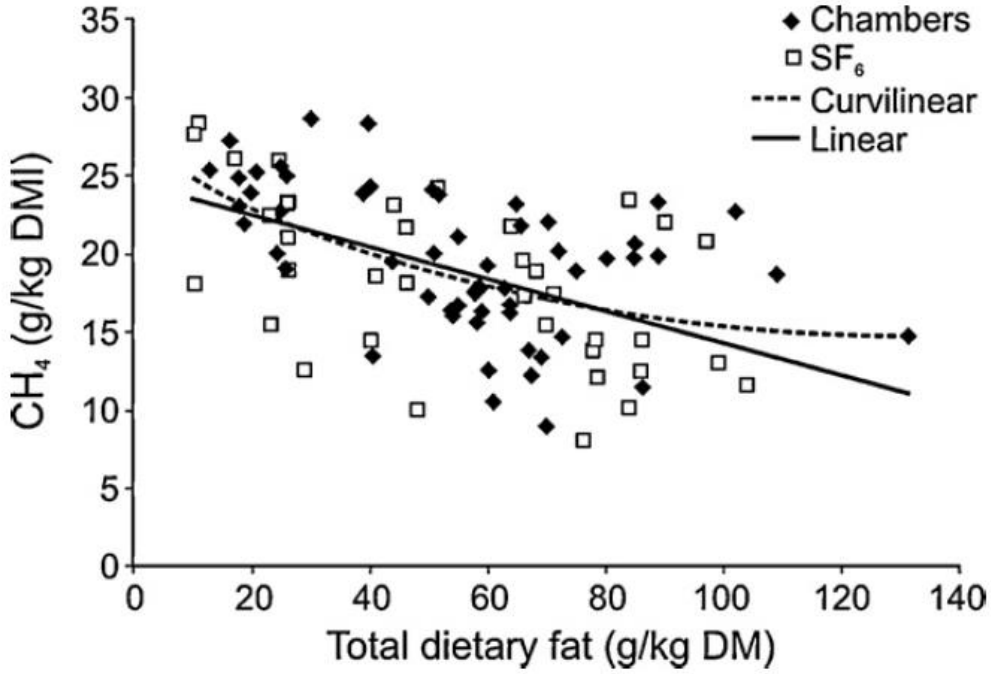


Figure 2. The effects of increasing dietary nitrate in ruminant animals on enteric methane emission responses; Y = 41.3 × nitrate (g kg^{-1} BW d^{-1}) + 1.2; R^2 = 0.76, *P* < 0.001 (Figure adapted from Lee and Beauchemin, 2014).

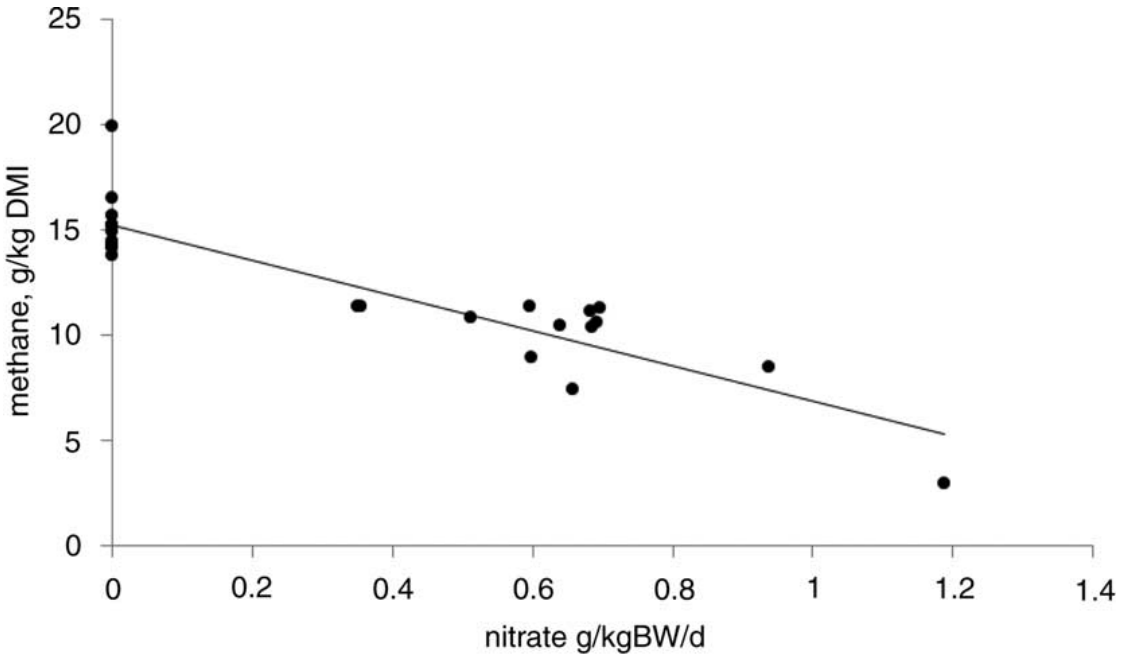


Figure 3. Total CH_4 emissions post-feeding in feedlot animals fed a high-forage diet supplemented with control, low (100 mg/kg), and high (200 mg/kg) doses of 3-nitrooxypropanol; $P < 0.01$ (Adapted from Vyas et al., 2016a).

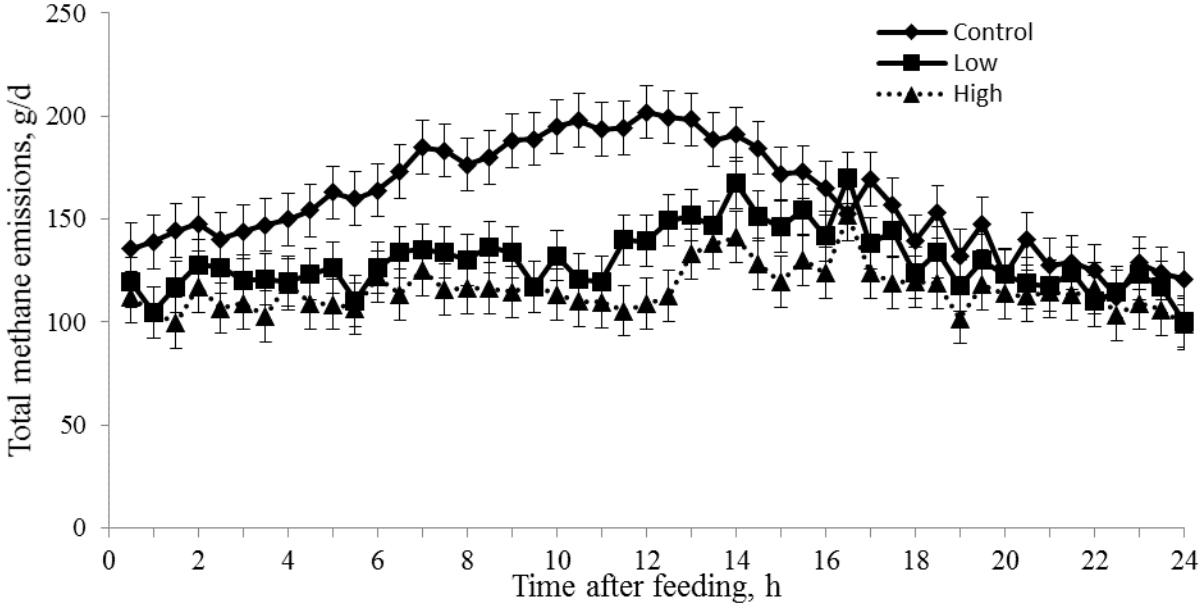
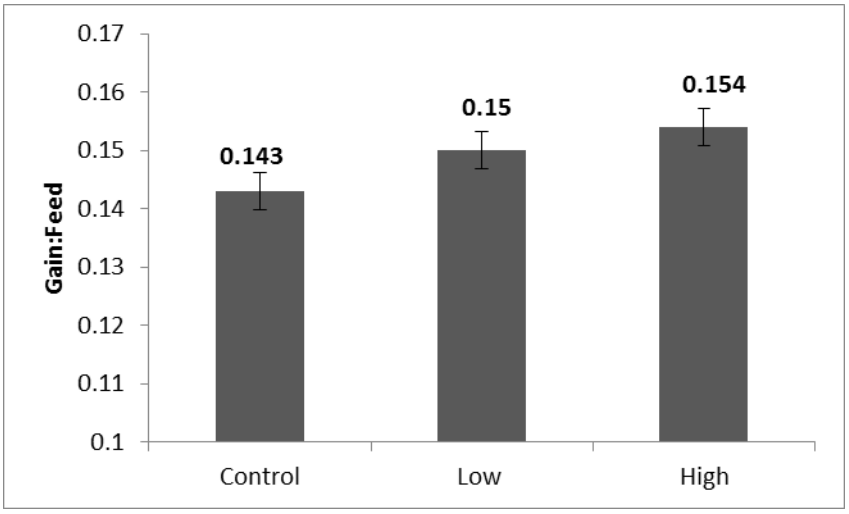


Figure 4. Gain-to-feed ratio and ADG in feedlot animals fed high-forage diets supplemented with control, low (100 mg/kg of DM), and high (200 mg/kg of DM) doses of 3-nitrooxypropanol; $P = 0.06$ (Vyas et al., 2016a).



Economics and Effects of Accelerated Calf Growth Programs

A. J. Heinrichs[1] and S .L. Gelsinger
Department of Animal Science, The Pennsylvania State University

Introduction

Feeding the dairy calf and heifer can be likened to a double-edged sword; we want to feed the heifers as much as possible to get rapid growth so that they begin lactating early in life, with a large body size at calving relative to their mature weight. However, there are issues related to rapid growth and a high level of feed intake that can go against the benefits and economics of such practices.

Growth and Development

As we look at dairy replacement growth, we know that the dairy heifer grows at its fastest rates in terms of body weight (**BW**) and skeletal growth from birth to puberty (Brody, 1945). For many of today's Holstein heifers, this rapid growth period extends to 8-10 months of age. At puberty, growth rates tend to decline on a percentage basis and composition of the growth shifts from predominately muscle and skeletal tissues to the accumulation of some fat (Brody, 1945).

The mammary gland also develops at a rapid rate during puberty and can be affected by animal growth rates during this time period (Tucker, 1987). Growth from weaning to puberty has been extensively studied, and a meta-analysis has shown that the optimal average daily gain (**ADG**) to grow a pre-pubertal heifer is about 1.75 lbs/d (800 g/d; Zanton and Heinrichs, 2005). At this stage heifers can gain 1.7 to 1.9 lbs/d with no appreciable losses in potential production.

Once puberty is reached, multiple data sets show that ADG does not affect milk production, as long as heifers reach an adequate size by the time they have their first calf. The goals are a BW of approximately 85% of mature BW and height at about 95% of mature stature. While data are less recent and discerning on this topic, there are supporting studies that show this effect (Fisher et al., 1983; Keown and Everett, 1986).

The digestive system of the calf is also maturing during the pre-weaning period, as the calf is maturing from a monogastric to a ruminant animal. The most notable change in the principal metabolic processes during ruminal development is the shift from a glycolytic to glucogenic liver (Baldwin et al., 2004). As the rumen begins to develop and microbial fermentation increases, less carbohydrate is available for postruminal digestion and the dietary supply of glucose diminishes. Research has shown that there is a substantially reduced rate of gluconeogenesis from lactate in

[1] Contact: 324 Henning Building, University Park, PA 16802, Work Phone: 814-863-3916, Email: ajh@psu.edu

ruminating calf liver cells, and data show a large decrease in the capacity to metabolize lactate to glucose as calves undergo rumen development (Baldwin et al., 2004). This transition results in tremendous metabolic ramifications to calf growth rate, as tissues must convert from reliance on glucose supplied from milk to the metabolism of short-chain fatty acids as primary energy substrates. Studies show that calves can effectively use propionate for glucose synthesis in the liver starting in early life (Donkin and Armentano, 1995). Once the rumen is developed, the calf can efficiently digest less costly starch- and fiber-based feedstuffs. While the most dramatic physical changes occurring during development are associated with the ruminal epithelium, changes in intestinal mass and metabolism are also happening in response to dietary changes. In addition, it has been shown that butyrate, an end product of ruminal digestion of starch, improves the development of small intestinal absorptive tissue (Gorka et al., 2011). To prepare the calf for weaning, it is important that the shift to ruminant digestion commence early in life and, once it begins, it needs to be developed at a reasonable rate to ensure efficient digestion and utilization of feedstuffs.

Factors Influencing Growth

Now, back to calf ADG as it relates to economics and production capability. If we look at what determines calf ADG, we know it is dry matter intake (liquid feeds, calf starter, and forage) and health (covering many issues that may affect the calf) (Place et al., 1998). A longitudinal calf growth study following heifers on 21 commercial farms from birth through multiple lactations (Heinrichs and Heinrichs, 2011) showed that dry matter intake at weaning positively affected first lactation milk production. Illness in the first 4 months of life had a negative effect on future milk production.

In a recent study looking at growth data across various calf nutrition experiments, the results suggest that pre-weaning growth rate is an important factor impacting future milk yield (Van De Stroet et al., 2016). After calving, heifers were categorized based on their weight and height as calves and their lactation performance was compared. In this analysis, calf starter was the primary source of differences in nutrient intake, since milk replacer was constant between the studies compared. This study showed that calves of shorter stature produced less milk in their first lactation after accounting for BW differences in the first lactation. Animals with medium BW as calves produced more milk in early lactation than those with high BW as calves, after accounting for differences in height. Calves that grew more quickly, ate more, and weighed more were heavier as first-lactation cows and as mature cows. Calves with the shortest stature had the lowest milk production potential and were the least likely to remain in the herd until first lactation. Pre-weaning ADG may be indicative of metabolic efficiency; therefore, it is possible that metabolically efficient calves continue to be metabolically efficient as adults (Van De Stroet et al., 2016).

Growth Rate and Future Milk Production

Feeding rate or nutrient intake has also been indicated as a factor that may influence first lactation milk production. In the past 5-10 years, there has been a trend

for feeding more milk or milk replacer due to accounts that this practice not only supplies more nutrients needed for rapid growth, but also may allow the animal to produce more milk in their first lactation. Multiple studies have addressed this question. A recent meta-analysis (Gelsinger et al., 2016) shows results from peer-reviewed research published in the past 20 years that measured the effect of milk or milk replacer intake, calf starter intake, and ADG before weaning on milk production from those calves in their first lactation (**Table 1**). While individual papers generally concluded that there was no effect, combining them in a meta-analysis revealed some additional information. While the results did show a positive impact of ADG on first-lactation milk production, it is important that we note the overall influence of ADG as a factor affecting production was small. The calf feeding program accounted for less than 3% of the variation in first-lactation milk yield within these studies. There are many factors that can affect the health and growth of heifers and their performance in the milking herd. Regardless, feeding program did have some impact, and the importance of feeding starter along with milk or milk replacer was evident. Increasing dry matter intake from milk or milk replacer by 0.2 lb/d (100 g/d) resulted in 145 lbs (66 kg) more milk in the first lactation. The same increase in milk or milk replacer resulted in 585 lbs (139 kg) more milk when combined with a 0.2 lb/d (100 g/d) increase in intake of calf starter.

These results emphasize the importance of providing readily available energy and protein in a liquid diet alongside a fermentable solid feed that can provide the end products and nutrients necessary to stimulate rumen development. It is important to ensure that nutrient requirements for maintenance, growth, and rumen development are met within the confines of calves' intake capacity.

One of the great advantages of pre-ruminant calves is their efficiency at converting nutrients to growth. While research confirms that increasing growth rate prior to weaning can improve milk production, there are two important questions to consider before setting out to maximize growth. First, will the expected increase in milk production offset the cost of the increased milk or milk replacer necessary to achieve high rates of growth? With the ever-increasing price of high-quality proteins used in milk replacers, this is especially pertinent for farms that feed milk replacer.

Consider the example of moving your calves from an average growth rate of 1.1 lb/d to 1.3 lb/d using the previously described meta-analysis (Gelsinger et al., 2016). We used NRC values and based the comparison on a typical milk-based, 20:20 milk replacer (**Table 2**). Capturing an extra 0.2 lb/d of ADG would require feeding an additional 12.8 lb of a 20:20 milk replacer, 12.3 lb of an accelerated milk replacer(27:17), or 10.4 gallons of milk over an 8-week pre-weaning period. Assuming $80 and $100 per 50-lb bag of 20:20 or accelerated milk replacer, respectively, and a milk price of $18/cwt, the cost of increasing growth from 1.1 to 1.3 lb/d is $20.45 (20:20), $24.53 (27:17), or $16.14 (saleable milk) per calf (**Table 3**). If a farm can feed all of their calves on 100% waste milk (valued at $4.50/cwt), the cost decreases to $4.04/calf. In contrast, the expected increase in milk income from these heifers is $3.09/heifer. This example assumes the milk price doesn't change in the two years it takes to get the heifer from weaning to calving.

Next we will consider the economics of using starter feed to increase pre-weaning growth rates (**Table 4**). In this case we assumed that there was sufficient milk being fed to meet maintenance needs of the calf and that the additional calf starter will go only towards growth. We do not have data separating maintenance from gain using starter in young calves, nor would it be realistic to only feed starter. Using the same growth comparisons and NRC data, we made similar comparisons. Achieving those gains is far less expensive due to the cost differences between milk products and calf starter (plus these gains do not account for maintenance). The change in production and value of the increased milk production is the same, but it costs far less to achieve these gains and actually can show a positive return if the gain is from 1.5 to 2.0 lbs/d since the return is roughly a 2 to 1 rate. This comparison also assumes that increasing calf growth rate does not change age at breeding or age at first calving, which could have dramatic economic benefits. Obviously feeding more to calves will cost money, but the comparison shows that grain feeding is far less costly than milk feeding and the ADG outcomes are the same, with the exception that feeding grains will increase ruminant digestion and intestinal development. Increasing heifer growth rates, regardless of the feeding strategy, will increase the possibility of decreasing age at calving, which can dramatically decrease heifer costs.

Conclusions

We conclude that gains in first-lactation production accomplished by increasing calf ADG pre-weaning are small and account for less than 3% of the variation in first-lactation milk production. Genetics, health, and other farm management practices will account for 97% of the actual milk production that we observe. Furthermore, any improved ADG that we want to accomplish in pre-weaned calves is far cheaper to do by increasing calf starter intakes in combination with a reasonable milk/milk replacer program.

References

Baldwin, R. L., VI, K. R. Mcleod, J. L. Klotz, and R. N. Heitmann. 2004. Rumen Development, Intestinal Growth and Hepatic Metabolism In The Pre- and Postweaning Ruminant. J. Dairy Sci. 87:E55-E65.

Brody, S. 1945. Bioenergetics and Growth. Waverly Press, Baltimore, MD.

Donkin, S. S., and L. E. Armentano. 1995. Insulin and glucagon regulation of gluconeogenesis in preruminating and ruminating bovine. J. Anim Sci. 73:546-551.

Fisher, L. J., J. W. Hall, and S. E. Jones. 1983. Weight and age at calving and weight change related to first lactation milk yield. J. Dairy Sci. 66:2167-2172.

Gelsinger, S. L., A. J. Heinrichs, and C. M. Jones. 2016. A meta-analysis of the effects of preweaned calf nutrition and growth on first-lactation performance. J. Dairy Sci. 99:6206-6214.

Gorka, P., Z. M. Kowalski, P. Pietrzak, A. Kotunia, W. Jagusiak, and R. Zabielski. 2011. Is rumen development in newborn calves affected by different liquid feeds and small intestine development? J. Dairy Sci. 94:3002-3013.

Heinrichs, A. J., and B. S. Heinrichs. 2011. A prospective study of calf factors affecting first-lactation and lifetime milk production and age of cows when removed from the herd. J. Dairy Sci. 94:336-341.
Keown, J. F., and R. W. Everett. 1986. Effect of days carried calf, days dry, and weight of first calf heifers on yield. J. Dairy Sci. 69:1891-1896.
Place, N. T., A. J. Heinrichs, and H. N. Erb. 1998. The effects of disease, management, and nutrition on average daily gain of dairy heifers from birth to four months. J. Dairy Sci. 81:1004-1009.
Tucker, H. A. 1987. Quantitative estimates of mammary growth during various physiological states: A review. J. Dairy Sci. 70:1958-1966.
Van De Stroet, D. L., J. A. Calderon Diaz, K. J. Stalder, A. J. Heinrichs, and C. D. Dechow. 2016. Association of calf growth traits with production characteristics in dairy cattle. J. Dairy Sci. 99:8347-8355.
Zanton, G. I., and A. J. Heinrichs. 2005. Meta-analysis to assess effect of prepubertal average daily gain of Holstein heifers on first-lactation production. J. Dairy Science. 88:3860-3867.

Table 1. Summary of studies included in the meta-analysis by Gelsinger et al. (2016).

Study	Comparison	Effect on first-lactation milk production [1]
Castells et al., 2015	Milk replacer with vs without oat hay supplementation	No difference
Kiezebrink et al., 2015	Whole milk feeding at 4 L/d vs 8 L/d	No difference
Margerison et al., 2013	Whole milk only at 4 L/d vs whole milk (4 L/d) with supplemental plant carbohydrates vs whole milk (4 L/d) with supplemental plant carbohydrates and amino acids	Greater in supplemented animals
Davis Rinker et al., 2011	Low vs high milk replacer feeding rate	No difference
Moallem et al., 2010	Conventional milk replacer vs whole milk	Greater in animals fed whole milk
Morrison et al., 2009 [2]	5 L/d vs 10 L/d of milk replacer	No difference
Raeth-Knight et al., 2009	Conventional milk replacer vs various intensive feeding programs	No difference
Terré et al., 2009	Low vs high milk replacer feeding rate	No difference
Shamay et al., 2005	Conventional milk replacer vs whole milk	No difference

[1] Treatment effects declared at $P < 0.05$.
[2] Morrison et al. (2009) also compared high and low milk replacer protein content; however, this comparison was not included in the current analysis.

Table 2. Effect of preweaning growth rate on metabolizable energy (ME) requirement during the preweaning period and predicted milk yield in first lactation.

	Preweaning growth rate (lb/d)				
	1.1	**1.3**	**1.5**	**1.8**	**2.0**
Birth weight (lbs)	99	99	99	99	99
Weaning weight (lbs)	161	173	185	198	210
Average preweaning body weight (lbs)	130	136	142	148	154
ME requirement for growth					
Daily (Mcal/d)	1.55	1.97	2.40	2.87	3.35
Total for 8 weeks (Mcal)	87.07	110.17	134.65	160.45	187.50
Total ME requirement					
Daily (Mcal/d)	3.68	4.17	4.68	5.22	5.77
Total for 8 weeks (Mcal)	206.3	233.6	262.3	292.2	323.3
Estimated 1st lactation 305-d milk yield (lbs)	26,581	26,599	26,638	26,701	26,786

Table 3. Estimated feed cost and value of additional milk produced in the first lactation if preweaning growth rate was increased by feeding more milk or milk replacer.

	Change in growth rate (lb/d)		
	1.1 to 1.3	**1.1 to 1.5**	**1.5 to 2.0**
Increased feed cost to support higher growth rate			
"Cheap" milk replacer ($80/50 lbs)	$20.45	$41.92	$45.73
"High quality" milk replacer ($100/50 lbs)	$24.53	$50.26	$54.83
Saleable milk ($18/cwt)	$16.14	$33.08	$36.09
Waste milk ($4.50/cwt)	$4.04	$8.27	$9.02
Estimated change in milk yield (lbs/lact)	17.2	57.0	147.7
Value of additional milk ($18/cwt)[1]	$3.09	$10.26	$26.58
Additional milk value minus increased feed cost[2]			
"Cheap" milk replacer ($80/50 lbs)	($17.36)	($31.66)	($19.15)
"High quality" milk replacer ($100/50 lbs)	($21.44)	($40.00)	($28.25)
Saleable milk ($18/cwt)	($13.05)	($22.82)	($9.51)
Waste milk ($4.50/cwt)	($0.95)	$1.99	$17.56

[1] Assumes same value for milk that is fed and milk that is sold.

[2] Does not include possible benefits from earlier age at first breeding/calving.

Table 4. Estimated feed cost and value of additional milk produced in the first lactation if preweaning growth rate was increased by feeding more calf starter[1,2]

	Change in growth rate (lb/d)		
	1.1 to 1.3	**1.1 to 1.5**	**1.5 to 2.0**
Total calf starter for higher growth rate (lbs/calf/d)	2.63	3.22	4.48
Additional calf starter (lbs/calf/56 d)	30.8	63.8	70.6
Cost of calf starter ($/calf)	$5.56	$11.45	$12.72
Estimated change in milk yield (lbs/lact)	17.2	57	147.7
Value of additional milk ($18/cwt)	$3.09	$10.26	$26.58
Value of additional milk minus cost of calf starter ($/calf)	($2.47)	($1.19)	$13.86

[1] Calf starter assumptions: 88% DM, 18% CP, 3.28 Mcal/kg, 57% available nutrients; cost $0.18/lb.

[2] Assuming all maintenance requirements are met by milk or milk replacer and all growth requirements are met by calf starter.

Modeling the effects of liquid intake and weaning on digestibility of nutrients in pre- and post-weaned dairy calves

J. D. Quigley[1], T. M. Hill, F. X. Suarez-Mena, T. S. Dennis, J. M. Aldrich, and R. L. Schlotterbeck

Nurture Research Center, Provimi North America, Cargill Premix and Nutrition

Introduction

Accurate predictions of nutrient supply and nutrient requirements are essential to modern ration formulations and animal production. Accurate and precise models allow provision of nutrients to meet requirements for maintenance and optimal production without supplying excess nutrients that contribute to inefficiency or environmental damage.

Most nutrient models predict supply of metabolizable energy (**ME**) and metabolizable protein (**MP**); in lactation models, flow of nutrients are predicted from endogenous, microbial, and undegraded dietary sources. Nutrient requirements are usually predicted using factorial calculation of requirements for maintenance (adjusted for environmental and management considerations), growth, pregnancy, and lactation. Only maintenance and growth predictions are used to predict nutrient requirements for calves, with requirements for pregnancy included for primiparous heifers.

For young calves and heifers, prediction of nutrient supply predicted by the 2001 Nutrient Requirements of Dairy Cattle (NRC, 2001) assume fixed digestibility and metabolizability of energy and protein. For example, calculation of ME from milk replacer is assumed to be the caloric content of protein, fat, and lactose adjusted for digestibility and metabolizability:

ME (Mcal/kg) = [(0.057 x CP) + (0.092 x EE) + (0.0395 x CHO)] x 97% x 96%, where:

CP = crude protein %, EE = ether extract %, CHO = carbohydrate % and 97% = digestibility of nutrients and 96% = metabolizability of digested nutrients.

Metabolizable energy content of calf starters is calculated as the sum of the digestible fractions of protein, non-fiber carbohydrates, neutral detergent fiber (**NDF**), crude protein (**CP**), and fat as described in the 2001 Dairy NRC (NRC, 2001) for adult cattle. Neither liquid nor starter feeds are corrected for differences in digestibility caused by age or development of the gastrointestinal tract in these models.

In young calves, digestibility of dry feeds (concentrates and forages) depends on

[1] Contact: 10 Nutrition Way, Brookville, OH 45309, USA. (937) 770-2400. Email: Jquigley@provimi-na.com

development of ruminal fermentation and intestinal digestion. This is particularly true for NDF (primarily fermented in the rumen) and starch (dependent on ruminal fermentation and small intestinal digestion). Studies have shown that fiber fermentation is limited in neonatal calves (Chapman et al., 2016; Hill et al., 2016a, b). Further, pancreatic α-amylase production is low at birth (Siddons, 1968) but increases with age (Huber et al., 1961; Morrill et al., 1970) along with total pancreatic secretion (McCormick and Stewart, 1966) thereby affecting small intestinal digestion of starch (Morrill et al., 1970).

Development of microbial fermentation changes flow of nutrients from the stomach. Prior to weaning, nutrients are derived primarily from milk protein, fat, and lactose; after weaning, nutrients are provided by volatile fatty acids absorbed from the rumen and microbial protein that increases in flow with increasing dry feed intake (Leibholz, 1975; Quigley et al., 1985).

Changing amounts and types of liquid fed to calves may alter age at which dry feed intake begins (Hill et al., 2006a, b; Strzetelski et al., 2001) thereby altering rumen development. This is particularly true when large amounts of liquid are fed (i.e., greater than about 700 g of solids from liquid/day for Holstein calves) since large amounts of liquid consumed will delay rumen development (Terré, et. al, 2007). Several studies have reported increased BW at weaning for calves fed large amounts of liquid pre-weaning; however, the advantage in growth compared to conventional feeding methods (500-700 g of solids/day) may be lost as BW gain slows dramatically in the period immediately post-weaning. We have attempted to quantify the effects of increased milk replacer allowance on digestibility of starter and its effects on growth and efficiency of young calves to determine if differences in digestion of nutrients, but particularly of carbohydrates, which may be at least partially responsible for differences in growth.

Digestion of Solid Feed

Calves are commonly weaned between 1 and 3 months of age in most dairy systems, with the most common age being approximately 9 weeks of age in the U.S. (USDA, 2016). Weaning to dry feed requires that the calf has sufficient digestive and fermentative capability to provide nutrients to support maintenance and growth. Further, the source of nutrients changes from milk digested primarily in the small intestine to grain-based ingredients fermented in the rumen and (or) digested in the small intestine. Therefore, gastrointestinal, hepatic, and systemic enzyme systems must be sufficiently adapted to changing sources of nutrients. If a calf is inadequately prepared for weaning, performance may suffer and predispose calves to reduced growth, poor efficiency, and even increased susceptibility to disease (Roth et al., 2008, 2009).

The most important factor in promoting rumen development and adaptation in preparation for weaning is consumption of dry feed containing fermentable carbohydrates – particularly sugars and starch – that are fermented to propionate and butyrate in the rumen by resident rumen bacteria. Production of volatile fatty acids and microbial protein stimulate a series of adaptations in the rumen, gastrointestinal tract,

hepatic tissues, and systemically that promote gluconeogenesis, production and release of β-hydroxybutyrate by ruminal epithelium, and utilization of acetate by peripheral tissues (Howarth et al., 1968; Huber, 1969; Baldwin et al., 2004).

In the past 15 years, some dairy experts have recommended feeding milk or milk replacer in excess of the traditional recommendations (approximately 10% of body weight as milk or reconstituted milk replacer) to increase rate of gain and take advantage of improved calf efficiency (Diaz et al., 2001; Davis-Rincker et al., 2011; Moallem et al., 2010). High digestibility and metabolizability of liquid feeds compared to higher fiber ingredients in calf starters naturally contributes to greater efficiency of BW gain.

Calves fed whole milk for *ad libitum* consumption or milk replacer to amounts >1 kg of powder per day gain impressive amounts of BW. For example, Jasper and Weary (2002) reported that calves fed milk for *ad libitum* consumption were 8 kg heavier at the end of a 63-d feeding period compared to calves fed milk at 10% of BW. All calves were weaned at 42 d. However, daily BW gains in calves fed for *ad libitum* consumption were markedly lower during the week of weaning (0.36 vs. 0.53 kg) and after weaning (0.68 vs. 0.85 kg) so that BW differences at 63 d were not as great as the difference prior to weaning.

Differences in growth rate post-weaning in calves fed differently pre-weaning may be due to differences in gastrointestinal development and digestion. Several recent studies indicate that digestion of nutrients from dry feeds varies when calves are fed varying amounts of liquid pre-weaning.

Terré et al. (2007) fed Holstein bull calves (19 d of age at start of the trial) milk replacer (**MR**) at levels typical of conventional feeding (**CF**; 4 L/d with weaning at 35 d of the study) or an enhanced feeding (**EF**) program wherein amount of MR was increased to 7 L/d and then reduced at weaning.

Total starter intake on the CF and EF programs prior to weaning were 23.8 and 12.6 kg, respectively. Results of a digestion trial conducted during d 38-42 of the study are in **Table 1**. These data indicate clearly that digestion of dry feed was impaired in calves fed EF, likely due to inadequate rumen development as a result of lower starter intake.

Digestion of NDF (derived primarily from wheat middlings, soybean hulls, and wheat distiller's grains) in the study by Terré et al. (2007) was lower in EF calves compared to CF calves (20.3 vs. 34.7%; **Table 1**). Since disappearance of NDF is due primarily to ruminal fermentation, it is likely that reduced NDF digestion was due to inadequate or incomplete ruminal fermentation in EF calves. Reduced NDF digestibility occurred in EF calves in spite of a higher rumen pH (5.73 vs. 5.99). Ruminal pH values less than approximately 6.0 are associated with impaired ruminal fiber fermentation (Allen, 1997; Shriver et al., 1986) due to pH sensitivity of cellulolytic bacteria in the rumen (Hoover, 1986; Russell et al., 1996). In the study by Terré et al. (2007), the

authors attributed higher ruminal pH to lower ruminal activity due to lower starter intake and a lack of substrate available for fermentation.

Leibholz (1975) monitored digestion of nutrients in calves fed whole milk or MR to weaning at 35 d of age. After weaning, calves were offered a pelleted feed consisting of 58% barley, 20% soybean meal, 15% wheat straw, and 3% molasses plus vitamins and minerals. The diet contained 15% protein and 13% ADF; we estimated the diet contained 2.7 Mcal of ME/kg and 50% non-fiber carbohydrate. By 6 wk of age (1 wk post-weaning), digestibility of ADF reached 57% and did not change markedly thereafter. However, the site of ADF digestion changed dramatically with time after weaning as most ADF was digested in the hindgut during the first 4 wk of the trial (**Figure 1**). Weekly DMI for each week of the 8 wk study were 0.6, 1.1, 1.5, 2.1, 2.2, 2.4, 2.5, and 2.5 kg/d. Intake of ADF ranged from 77 g/d in the 1st week post-weaning to 325 g/d at wk 8. Therefore, it is possible that higher digestion of ADF in the hindgut during the first few weeks after weaning was due to small amounts of ADF consumed.

Hill et al. (2010) fed calves (2-3 d of age at start of study) one of four MR programs: 0.44 kg of DM of a 21% CP, 21% fat MR powder fed daily for 42 d (**A**); 0.66 kg of DM of a 27% CP, 17% fat MR powder fed daily for 42 d (**B**); 0.66 kg of DM of a 27% CP, 17% fat MR powder daily fed for 28 d (**C**); or up to 1.09 kg of DM of a 29% CP, 21% fat MR daily fed for 49 d (**D**). Digestibility estimates were made on d 53 to 56. **Table 2** shows clearly that digestion of dry matter (**DM**) and organic matter (**OM**) were lower when calves were fed large amounts of MR prior to weaning (treatment D). During the digestibility period (d 53 to 56), intake of starter DM was 2.2, 2.3, 2.5 and 1.9 kg/d for treatments A, B, C, and D, respectively. The trend ($P < 0.08$) for low starter DM intake coupled with significantly lower digestion of DM resulted in calves on treatment D only consuming about 71% of the digestible DM of calves on the other treatments.

More recently, Chapman et al. (2016) reported that digestion of nutrients, but particularly of NDF and ADF, were reduced during the digestion period of d 52-58 of age when calves were fed MR up to 0.87 kg/d (**Table 3**). Although digestion of all nutrients (except starch) were reduced significantly, digestion of NDF and ADF were reduced nearly 50% in calves fed large amounts of milk pre-weaning.

Conversely, Chapman et al. (2017) reported no difference in NDF digestion when calves were fed MR at 446, 669, or 892 g/d during the digestibility measurement period. Further, NDF digestion was 58, 69, and 69%, respectively, suggesting extensive digestion of fiber by the calves. However, the starter used in the study contained only 16% NDF and starter intake during the trial was 1.1, 0.7 and 0.4 kg/d, respectively. Measurements were taken prior to weaning, which may have increased the error associated with measurement.

A majority of these data suggest that calves fed large amounts of milk pre-weaning may have difficulty digesting nutrients from dry feed during the immediate post-weaning period. There are numerous implications to these findings. For example, digestion of starters containing greater amounts of fibrous by-products may be difficult if calves are

fed large amounts of liquid pre-weaning. Also, it may be necessary to use increasingly complex liquid reduction strategies to ensure that starter intake (and digestibility) is adequate prior to weaning.

Because fiber digestion is primarily influenced by cellulolytic fermentation in the rumen, the low digestibility of ADF and NDF (**Table 3**) indicate that the rumen is less well developed in calves fed greater amounts of MR (Chapman et al., 2016). Also, fiber digesting microorganisms are established in the rumen more slowly than starch and sugar digesting microorganisms (Anderson et al., 1987). Finally, selection of ingredients that may negatively affect ruminal fermentation (e.g., inclusion of oil-containing ingredients) may also reduce total DM digestion (Hill et al., 2015).

To better understand the changes in NDF digestion with age and diet, Hill et al. (2016b) fed calves a moderate or aggressive milk replacer feeding program and monitored changes in nutrient digestion with advancing age. **Figure 2** shows changes in NDF digestion with advancing age. The effect of diet is clearly shown, as calves fed more milk (AGG in Figure 2) maintained lower NDF digestion throughout the three digestibility periods. Also, calves fed functional fatty acids and nutrients (NeoTec5g®, Provimi North America, Brookville, OH, USA) feed additive (MOD+ and AGG+ in Figure 2) had higher NDF digestion in periods 2 (42-46 d of age) and 3 (54 to 58 d of age). Previous studies (Guilloteau et al., 2009, 2010; Hill et al. 2007) have shown that feeding sodium butyrate (a component of NeoTec5g) improved fiber digestion in young calves.

Calves fed the moderate MR program (MOD in Figure 2) consumed more starter throughout the trial, which likely hastened rumen development and the ability of calves to digest NDF. In calves fed MOD, NDF digestion increased from approximately 15% at 19-23 d of age to approximately 35% by 51-56 d of age. Digestion of NDF in calves fed the higher level of MR (AGG) did not change markedly through the 56-d study and there were few differences with advancing age.

In addition to age of calf, digestion of nutrients post-weaning is affected by ingredient source and form of calf starter. Digestion of DM, OM, and CP were higher in starters containing ground corn, whereas ADF and NDF digestion were greatest in starters containing soybean hulls (**Table 4**). Hill et al. (2016a) also reported that texturized calf starters containing whole corn and whole oats (51-54% starch and 13% NDF) had higher DM, OM, and CP digestibility than pelleted starters containing wheat middlings, soybean hulls, and dried distiller's grains (20% starch and 36% NDF; **Table 5**). On the other hand, pelleted, high-fiber starters had higher ADF, NDF, starch, and fat digestion. Gain of BW and hip width increased as OM digestibility increased in these trials.

Collectively, these data suggest that the availability of energy from starters is dependent on type of carbohydrate, form of the starter (texturized vs. pelleted), age of the calf, and intake of liquid pre-weaning.

Current nutrient models for calves and heifers (e.g., 2001 Dairy NRC) ignore the effects of previous nutrition and extent of rumen development. The ME content of starters is a static calculation based on expected digestibility of nutrient fractions (NDF, non-fiber carbohydrate, protein, and fat). No provision is made for differing nutrient digestibilities with advancing age or intake. Conversely, other models for lactating cows utilize dynamic calculations of energy based on rates of ruminal digestion of each fraction (NFC, NDF, protein, fat) and rate of passage (Higgs et al., 2015). Intestinal digestibility coefficients are then applied to the ruminally undegraded fraction to estimate total nutrient supply.

Using data from Chapman et al. (2016) and Hill et al. (2016b), we estimated ME concentrate of calf starter using the method outlined in the 2001 NRC Nutrient Requirements of Dairy Cattle (NRC, 2001) as well as calculated ME based on analyzed values using digestibility data from Table 3 and Figure 2. Results are in **Table 6**. The column labeled “NRC” contains calculated ME concentration in starter based on the 2001 NRC method assuming digestibility values typical for adult ruminants. The column “Calculated” contains data using total tract digestibility measured in the studies by Chapman et al. (2016) and Hill et al. (2016b). We also used the 2001 Dairy NRC model to predict ME-allowable BW gain using the ME values calculated for calf starter using the NRC (NRC ME-g) or calculated values (Calc. ME-g) in Table 6.

Differences were significant for all measurements, but ME was markedly overestimated in calves fed higher levels of milk in both studies. Consequently, predicted ME-allowable gains using the calculated ME value for calf starter were lower compared to predicted gains using the ME values calculated with the NRC calculations.

The implications of errors in calculation of ME content are clear, as calves fed high levels of milk pre-weaning will be ill prepared for weaning and will be unable to extract nutrients from calf starters efficiently. Consequently, growth of calves will be compromised until sufficient maturation of the digestive tract and associated tissues allows the calf to fully utilize nutrients in the calf starter. The existing NRC model over-predicts ME supply from starters by 12 to 26% (Table 6).

These data also suggest that additional time may be needed for a weaning transition to ensure that calves fed high levels of milk will consume sufficient starter prior to weaning. In most of the studies cited in this review, liquid intake was reduced for 7-10 d prior to weaning. For calves fed 1 kg of powder or greater, this is probably insufficient time for adaptation.

Conclusions

The 2001 Dairy NRC represented an important improvement in our understanding of nutrient requirements for young calves and heifers. Further refinement of methods to estimate nutrient supply of young calves will improve our ability to calculate growth under a wide range of feeding and management conditions.

Feeding varying amounts of liquid from milk or MR has important implications to growth post-weaning. Increasing liquid consumption above approximately 650-700 g of solids per day will delay initiation of calf starter intake and will delay onset of rumen development. Digestion of all nutrients, but particularly NDF, is essential to ensure that rumen development is adequate prior to weaning.

References

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

Anderson K. L., T. G. Nagaraja, J. L. Morrill, T. B. Avery, S. J. Galitzer and J. E. Boyer. 1987. Ruminal microbial development in conventionally or early-weaned calves. J. Anim. Sci. 64:1215-1226.

Baldwin VI, R. L., K. R. McLeod, J. L. Klotz, and R. N. Heitmann. 2004. Rumen development, intestinal growth and hepatic metabolism in the pre- and postweaning ruminant. 87(Suppl.):E55–E65.

Chapman, C. E., P. S. Erickson, J. D. Quigley, T. M. Hill, H. G. Bateman II, F. X. Suarez-Mena, and R. L. Schlotterbeck. 2016. Effect of milk replacer program on calf performance and digestion of nutrients with age of the dairy calf. J. Dairy Sci. 99:2740–2747.

Chapman, C. E., T. M. Hill, D. R. Elder, and P. S. Erickson. 2017. Nitrogen utilization, preweaning nutrient digestibility, and growth effects of Holstein dairy calves fed 2 amounts of a moderately high protein or conventional milk replacer. J. Dairy Sci. 100:279–292.

Davis Rincker, L. E., M. J. VandeHaar, C. A. Wolf, J. S. Liesman, L. T. Chapin, and M. S. Weber Nielsen. 2011. Effect of intensified feeding of heifer calves on growth, pubertal age, calving age, milk yield, and economics. J. Dairy Sci. 94:3554–3567.

Diaz, M. C., M. E. Van Amburgh, J. M. Smith, J. M. Kelsey, and E. L. Hutten. 2001. Composition of growth of Holstein calves fed milk replacer from birth to 105-kilogram body weight. J. Dairy Sci. 84:830–842.

Guilloteau, P., G. Savary, Y. Jaguelin-Peyrault, V. Romé, L. Le Normand, and R. Zabielski. 2010. Dietary sodium butyrate supplementation increases digestibility and pancreatic secretion in young milk-fed calves. J. Dairy Sci. 93:5842–5850.

Guilloteau, P., R. Zabielski, and J. W. Blum. 2009. Gastrointestinal tract and digestion in the young ruminant: Ontogenesis, adaptations, consequences and manipulations. J. Physiol. and Pharmacol. 60(Suppl. 3):37-46.

Higgs, R. J., L. E. Chase, D. A. Ross, and M. E. Van Amburgh. 2015. Updating the Cornell Net Carbohydrate and Protein System feed library and analyzing model sensitivity to feed inputs. J. Dairy Sci. 98:6340–6360.

Hill, T. M., J. M. Aldrich, R. L. Schlotterbeck, and H. G. Bateman II. 2006a. Effects of feeding calves different rates and protein concentrations of twenty percent fat milk replacers on growth during the neonatal period. Prof. Anim. Sci. 22:252–260.

Hill, T. M., J. M. Aldrich, R. L. Schlotterbeck, and H. G. Bateman II. 2006b. Effects of feeding rate and concentrations of protein and fat of milk replacers fed to neonatal calves. Prof. Anim. Sci. 22:374–381.

Hill, T. M., J. M. Aldrich, R. L. Schlotterbeck, and H. G. Bateman II. 2006b. Effects of feeding rate and concentrations of protein and fat of milk replacers fed to neonatal calves. Prof. Anim. Sci. 22:374–381.
Hill, T. M., H. G. Bateman II, J. M. Aldrich, and R. L. Schlotterbeck. 2010. Effect of milk replacer program on digestion of nutrients in dairy calves. J. Dairy Sci. 93:1105–1115.
Hill, T. M., H. G. Bateman II, J. M. Aldrich, J. D. Quigley, and R. L. Schlotterbeck. 2015. Inclusion of tallow and soybean oil to calf starters fed to dairy calves from birth to four months of age on calf performance and digestion. J. Dairy Sci. 98:4882–4888.
Hill, T. M., J. D. Quigley, H. G. Bateman II, J. M. Aldrich, and R. L. Schlotterbeck. 2016a. Source of carbohydrate and metabolizable lysine and methionine in the diet of recently weaned dairy calves on digestion and growth. J. Dairy Sci. 99:2788–2796.
Hill, T. M., J. D. Quigley, F. X. Suarez-Mena, H. G. Bateman II, and R. L. Schlotterbeck. 2016b. Effect of milk replacer feeding rate and functional fatty acids on dairy calf performance and digestion of nutrients. J. Dairy Sci. 99:6352–6361.
Hoover, W. H. 1986. Chemical factors involved in ruminal fiber digestion. J. Dairy Sci. 69:2755-2766.
Howarth, R. E., R. L. Baldwin, M. Ronning. 1968. Enzyme activities in liver, muscle, and adipose tissue of calves and steers. J. Dairy Sci. 51:1270-1274.
Huber, J. T., N. L. Jacobson, and R. S. Allen. 1961. Digestive enzyme activities in the young calf. J. Dairy Sci. 44:1494-1501.
Huber, J. T., 1969. Development of the digestive and metabolic apparatus of the calf. J. Dairy Sci. 52:1303-1315.
Jasper, J. and D. M. Weary. 2002. Effects of ad libitum milk intake on dairy calves. J. Dairy Sci. 85:3054–3058.
Khan, M. A., H. J. Lee, W. S. Lee, H. S. Kim, S. B. Kim, K. S. Ki, J. K. Ha, H. G. Lee, and Y. J. Choi. 2007. Pre- and postweaning performance of Holstein female calves fed milk through step-down and conventional methods. J. Dairy Sci. 90:876–885.
Leibholz, J. 1975. The development of ruminant digestion in the calf. I. The digestion of barley and soya bean meal. Aust. J. Agric. Res. 26:1081-1091.
McCormick, R. J. and W. E. Stewart. 1966. Pancreatic secretion in the bovine calf. J. Dairy Sci. 50:568-571.
Moallem, U., D. Werner, H. Lehrer, M. Zachut, L. Livshitz, S. Yakoby, and A. Shamay. 2010. Long- term effects of ad libitum whole milk prior to weaning and prepubertal protein supplementation on skeletal growth rate and first-lactation milk production. J. Dairy Sci. 93:2639–2650.
Morrill, J. L., W. E. Stewart, R. J. McCormick and H. C. Fryer. 1970. Pancreatic amylase secretion by young calves. J. Dairy Sci. 53:72-78.
National Research Council. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Sci., Washington, DC.
Quigley, III, J. D., C. G. Schwab, and W. E. Hylton. 1985. Development of rumen function in calves: nature of protein reaching the abomasum. J. Dairy Sci. 68:694-702.
Roth, B. A., E. Hillmann, M. Stauffacher, and N. M. Keil. 2008. Improved weaning reduces cross-sucking and may improve weight gain in dairy calves. Appl. Anim. Behav. Sci. 111:251–261.

Roth, B. A., N. M. Keil, L. Gygax, and E. Hillmann. 2009. Influence of weaning method on health status and rumen development in dairy calves. J. Dairy Sci. 92:645-656.
Russell, J. B. and D. B. Wilson. 1996. Why are ruminal cellulolytic bacteria unable to digest cellulose at low pH? J. Dairy Sci. 79:1503-1509.
Shriver B. J., W. H. Hoover, J. P. Sargent, R. J. Crawford Jr., and W. V. Thayne. 1986. Fermentation of a high concentrate diet as affected by ruminal pH and digesta flow. J. Dairy Sci. 69:413-419.
Siddons, R. C. 1968. Carbohydrase activities in the bovine digestive tract. Biochem. J. 108:839-844.
Strzetelski, J., B. Niwinska, J. Kowalczyk, and A. Jurkiewicz. 2001. Effect of milk replacer feeding frequency and level on concentrate intake and rearing performance of calves. J. Anim. Feed Sci. 10:413-420.
Terré, M., M. Devant, and A. Bach. 2007. Effect of level of milk replacer fed to Holstein calves on performance during the preweaning period and starter digestibility at weaning. Livestock Sci. 110:82–88.
USDA. 2016. Dairy 2014, "Dairy Cattle Management Practices in the United States, 2014". USDA–APHIS–VS–CEAH–NAHMS. Fort Collins, CO. #692.0216.

Digestibility, %	CF	EF	SE	P
Dry matter	77.4	71.8	1.23	0.01
Organic matter	78.7	73.2	1.18	0.01
Crude protein	77.1	71.6	1.29	0.01
Neutral detergent fiber	34.7	20.3	3.79	0.02
Gross energy	75.6	69.8	1.25	0.01

Table 1. Apparent total tract digestibility of dry feed in calves fed 4 L/d of milk replacer (**MR**) at 12.5% DM dilution rate from d 1–28, and 2 L/d from d 29 to d 35 (**CF**) or MR at 18% DM dilution rate: 4 L/d from d 1–6, 6 L/d from d 7–13, 7 L/d from d 14–20, 6 L/d from d 21–28, and 3 L/d from d 29 to 35 (**EF**). Digestibility was measured the week after weaning. Adapted from Terré et al. (2007).

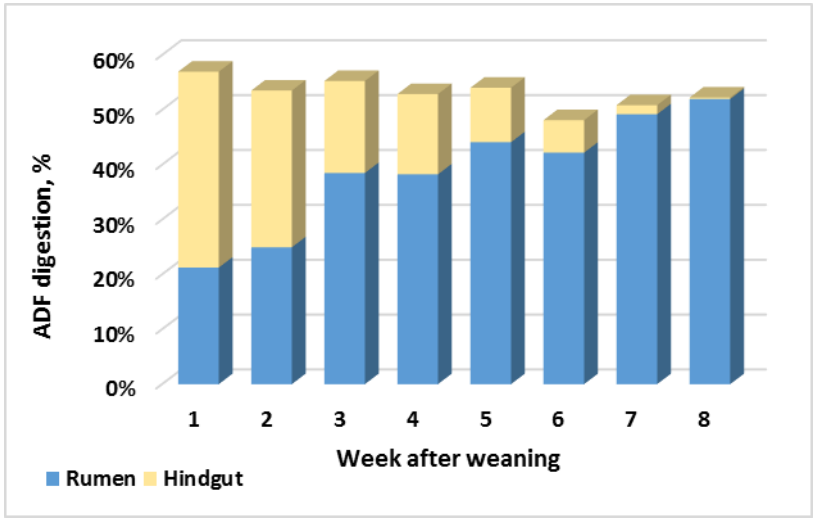


Figure 1. Digestion of acid detergent fiber in calves fed milk or milk replacer to weaning at 5 wk of age. Digestion was measured in the stomach and intestines using duodenally cannulated calves. Adapted from Leibholz, 1975.

Digestion, %	A	B	C	D	SE	P
DM	75.6[a]	78.3[a]	78.7[a]	67.3[b]	2.19	0.01
OM	77.4[a]	78.3[a]	78.7[a]	68.0[b]	2.20	0.01
CP	72.4	72.3	74.1	71.8	2.58	0.83
Fat	70.3	75.4	76.3	75.4	3.37	0.33

Table 2. Total tract apparent digestion of dry matter (**DM**), organic matter (**OM**), crude protein (**CP**) or fat in calves fed one of four MR programs: 0.44 kg of DM of a 21% CP, 21% fat MR powder fed daily for 42 d (**A**); 0.66 kg of DM of a 27% CP, 17% fat MR powder fed daily for 42 d (**B**); 0.66 kg of DM of a 27% CP, 17% fat MR powder daily fed for 28 d (**C**); or up to 1.09 kg of DM of a 29% CP, 21% fat MR daily fed for 49 d (**D**). Adapted from Hill et al., 2010.
[a,b]Means in the same row with different superscripts differ, $P < 0.05$.

Item	CON	MOD	AGG	SE	P
BW, kg	62.7[a]	72.3[b]	82.8[c]	4.05	0.01
DMI, kg/d	2.04	2.30	2.28	0.258	0.08
Digestibility, %					
DM	77.6[a]	76.9[a]	66.0[b]	1.67	0.01
OM	79.2[a]	78.2[a]	67.9[b]	1.65	0.01
ADF	56.3[a]	53.2[a]	26.7[b]	3.89	0.01
NDF	54.1[a]	50.7[a]	26.2[b]	2.86	0.01
Starch	96.7	94.5	94.0	1.33	0.36
CP	71.9[a]	74.1[a]	56.3[b]	2.72	0.02
Sugar	93.1[a]	91.5[a]	86.2[b]	1.68	0.02
Fat	81.4[a]	83.2[a]	74.1[b]	1.84	0.01

Table 3. Body weight (**BW**), DM intake (**DMI**) and total tract digestibility of nutrients in calves fed conventional [**CON**; 0.44 kg of dry matter (**DM**) 21% crude protein (**CP**), 21% fat powder fed for 42 d], moderate (**MOD**; 0.66 kg of DM 27% CP, 17% fat powder fed for 42 d), and aggressive program (**AGG**; up to 0.87 kg of DM 27% CP, 17% fat powder fed for 49 d). Digestibility was measured from d 51-56. From Chapman et al., 2016.

[a,b,c]Means in the same row with different superscripts differ, $P < 0.05$.

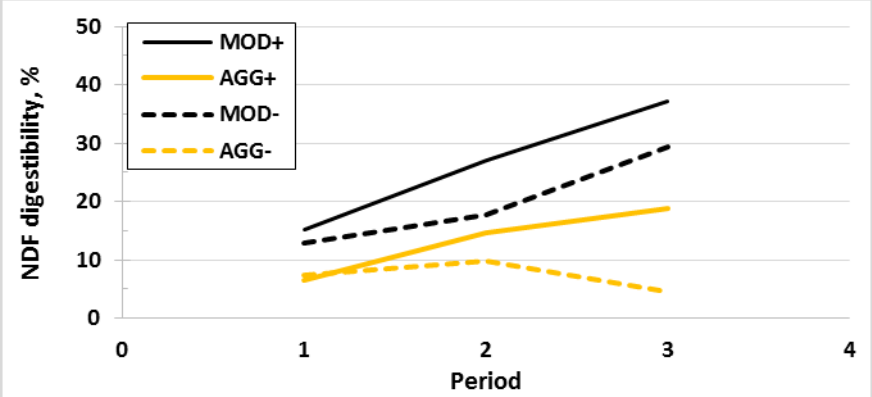


Figure 2. Change in total tract NDF digestibility in calves fed 0.66 kg of DM of a 27% CP, 17% fat MR powder daily fed for 49 d (**MOD**) without **(-)** or with **(+)** added NeoTec4 feed additive; or 0.66 kg of DM of a 27% CP, 17% fat MR powder fed for 4 d, then 0.96 kg of DM for 4 d, then 1.31 kg of DM fed for 34 d, then 0.66 kg of DM for 7 d (**AGG**). Effect of feeding level, NeoTec4 inclusion and age were significant ($P < 0.05$). Digestibility periods were 1 = 19-23 d; 2 = 40-44 d; and 3 = 52-56 d of the study. Calves were 2-3 d of age at initiation of the study. Adapted from Hill et al. (2016b).

Digestibility, %	S	M	C	SE	Contrast 1	Contrast 2
DM	76.9	78.9	85.2	1.58	0.01	0.23
OM	77.5	79.6	85.8	1.56	0.01	0.21
ADF	65.5	53.5	55.4	3.48	0.20	0.01
NDF	70.7	56.1	66.2	3.13	0.34	0.01
Starch	97.6	98.9	97.0	0.57	0.13	0.15
CP	78.1	80.7	84.4	1.75	0.01	0.16
Sugar	94.2	95.6	94.2	1.79	0.63	0.47
Fat	84.1	86.3	89.6	2.61	0.08	0.42

Table 4. Nutrient digestibility in calves 15-16 wk of age fed starters containing soybean hulls (S), wheat middlings (M) or corn (C). Contrast 1 = (S+M) vs. C; contrast 2 = S vs. M. Adapted from Hill et al., 2016a.

Digestibility, %	TX-MPL	TX-MPH	PL-MPL	PL-MPH	SEM	P
DM	84.3	84.7	79.7	78.8	0.51	0.001
OM	84.9	85.0	80.2	78.9	0.57	0.001
ADF	41.5	54.0	65.2	66.1	1.86	0.001
NDF	56.8	62.8	69.4	66.1	1.64	0.005
Starch	95.1	95.7	99.0	98.7	0.29	0.001
CP	84.9	84.6	79.5	78.6	0.54	0.001
Sugar	95.3	95.6	95.7	92.4	0.68	NS
Fat	86.3	82.7	88.3	87.8	0.78	0.08

Table 5. Nutrient digestibility in calves 15-16 wk of age fed high starch texturized (TX) or low starch pelleted (PL) starters containing low (MPL) or high MPH) amounts of metabolizable protein. No main effect of metabolizable protein was reported. P = probability of a main effect of starch level. Adapted from Hill et al., 2016a.

	Starter ME, Mcal/kg			Predicted ME grain, kg/d		
Item	**NRC**	**Calculated**	**%**	**NRC**	**Calc.**	**%**
Chapman et al. 2016						
CON	2.81	2.59	92	0.77	0.67	87
MOD	2.81	2.56	91	0.93	0.82	88
AGG	2.84	2.30	81	0.94	0.70	74
Hill et al. 2016b						
MOD-	2.81	2.52	90	0.83	0.71	86
AGG-	2.89	2.45	85	0.61	0.45	74
MOD+	2.83	2.60	92	0.77	0.68	88
AGG+	2.87	2.50	87	0.70	0.55	79

Table 6. Estimated ME concentration (Mcal/kg of DM) in calf starters used by Chapman et al. (2016) and Hill et al. (2016b) using methods of 2001 Dairy NRC (NRC) or calculated using total tract digestibilities reported in each experiment. ME-allowable BW gains were calculated using equations [2-4 a-e and 2-5 to 2-10] in 2001 Dairy NRC Requirements for Dairy Cattle (NRC, 2001) or using digestibility estimates from Table 3 and Figure 2, respectively. Digestibility estimates were made at 52-56 d.

The Role of the Small Intestine in Developmental Programming: Impact of Maternal Nutrition on the Dam and Offspring[1,2]

J. S. Caton[3] and A. M. Meyer[4]
[3]Center for Nutrition and Pregnancy, Department of Animal Sciences, North Dakota State University
[4]Division of Animal Sciences, University of Missouri, Columbia

Introduction

Small intestinal growth and function are critical for optimal animal growth and health, playing a major role in nutrient digestion and absorption, energy and nutrient expenditure, and immunological competence. Small intestinal growth and development are often overlooked but essential processes driving metabolism, immunology, survival, and growth. The small intestine not only serves as the main site for digestion and absorption of nutrients, but it is also a major energy and nutrient sink due to its high metabolic activity and rapid turnover. Changes in small intestinal mass, cellularity, and oxygen consumption have been demonstrated during feed restriction and in response to specific nutrients. The effects of in utero environment have become a major area of study in animal and human nutrition, physiology, and epidemiology research, as evidenced by the hundreds of reviews on the subject. In livestock, intrauterine growth restriction (**IUGR**) results in impaired fetal development, low birth weight offspring, and decreased long-term production. Programming of growth and development in livestock may be driven by many factors, but often occurs in response to compromised nutrient supply to developing offspring. Because the small intestine is critical to animal growth, health, and production and is responsive to its luminal and extraluminal environment, early life effects on small intestinal development likely play a significant role in observed programming of later animal health and performance, including the acquisition of nutrients during the pre- and postnatal periods. Additionally, impacts of gestational nutrition on the maternal small intestine may change nutrient delivery to offspring, both in utero and during lactation. This review will focus on impacts of nutrition during pregnancy on maternal and offspring small intestines and focus on data from ruminant livestock models.

Fetal Small Intestinal Growth and Development

There are multiple developmental windows (**Figure 1**) for the small intestine

---

[1] Portions of this article were previously published as a review paper (American Society for Nutrition. Adv, Nutr, 2016;7:169–178; doi:10.3945/an.115.010405) and presented here in accordance with granted author rights as outlined in policies of Adv. Nutr and American Society of Nutrition. Portions of the data were also presented at the symposium “Maternal/Fetal Nutrition and Programming: What Have We Learned from Farm Animal Models?” held 28 March 2015 at the ASN Scientific Sessions and Annual Meeting at Experimental Biology 2015 in Boston, MA.

[2] Contact: Department of Animal Sciences, North Dakota State University, Fargo, ND. 58108. Joel.Caton@ndsu.edu.

during fetal, perinatal, and neonatal periods. Organogenesis generally occurs during early to mid-gestation, followed by rapid fetal growth in the last third of gestation, then preparation for the transition from the uterine to the outside environment during the perinatal period. In addition to these windows, the small intestine continues to develop postnatally and even into maturity, when it remains plastic and responds to physiological state, diet, and other factors.

Evidence of Developmental Programming of the Offspring Small Intestine Intrauterine Growth Restriction

Effects of IUGR on the small intestine (**Table 1**) generally include reduced mass and/or length of the small intestine, decreased villus and crypt density, villus height and/or width, crypt depth, and mucosal size which suggest that reduced mass may also be accompanied by reduced functional area and development. Additional decreases in proliferation and cellular differentiation suggest altered crypt proliferative dynamics. Although effects of IUGR on the small intestine have been better characterized prenatally or immediately after birth, these effects persist postnatally.

Gene expression in the small intestine has also been altered by IUGR. Piglets identified as IUGR had altered jejunal protein expression, including 7 down-regulated and 4 up-regulated genes. Altered ileal gene expression was also observed in IUGR compared with normal piglets, although these were affected by day of sampling (birth vs. d 2 or 5 postnatally). At each time point, genes differentially expressed included those involved in macromolecule metabolism, biosynthesis, and cellular metabolism.

Although many of the reported effects of IUGR on the small intestine appear to be negative, this is not always the case. For example, jejunal lactase and maltase were greater for IUGR rats than control rats at birth, although this did not extend past the immediate postnatal period (Qui et al., 2005). These authors suggested that increased digestive enzyme production at birth was an adaptive mechanism allowing IUGR neonates to have increased digestive capacity. In another study, ileal adherent bacterial numbers were increased for IUGR pigs at d 2 postnatally (D'Inca et al., 2010), indicating that IUGR can alter bacterial colonization of the small intestine postnatally.

Maternal Nutrient Manipulation during Gestation

Research indicates that both maternal nutritional plane (**Table 2**) and specific nutrient intake can affect the fetal small intestine. Timing of these maternal nutritional insults is important due to the developmental windows outlined in **Figure 1**.

Fetal. Nutrient restriction during early and mid-gestation does not appear to impact fetal small intestinal growth. Nutrient restriction during early and mid-gestation can increase jejunal crypt proliferation at d 125 of gestation in fetal calves. Additionally, when nutrient-restricted cows were realimented, total vascularity of the fetal small intestine was increased at d 245 of gestation. These data suggest that nutrient restriction increased the efficiency of the fetal small intestine, perhaps similarly to the

“thrifty phenotype” hypothesis (Hales and Barker, 1992), which has been postulated to describe fetal development changes that increase survival in the face of a negative environment or poor nutrition (Wells, 2007).

Maternal nutrient restriction of ewes in mid- and late gestation has decreased small intestinal mass and jejunal hypertrophy (protein:DNA), despite a lack of differences in jejunal proliferation. Lambs from nutrient- restricted ewes had decreased total jejunal microvascular volume concurrently with reduced jejunal mRNA expression of soluble guanylate cyclase (**GUCY1B3**), a NO receptor involved in vasodilation and angiogenesis. Conversely, small intestinal mass of fetal lambs from ewes that were nutrient restricted during the last 3 wk of gestation was unaffected, suggesting that longer periods of maternal nutrient restriction are necessary to affect the fetal small intestine. Nutrient restriction during mid- and late gestation has increased oxygen consumption per unit of small intestine in late-term fetal lambs.

Postnatal. Changes in maternal nutrition in late gestation may negatively affect gut maturation. Cortisol and fetal swallowing of amniotic fluid both play an important role in the small intestinal maturation process (Sangild et al., 2000; Trahair and Sangild, 2004). For example, expression of vascular endothelial growth factor (**VEGF**) in the fetal small intestine, which is important for angiogenesis of the growing tissue, is likely cortisol-dependent in sheep (Holmes et al., 2008). Maternal cortisol levels are often changed by gestational plane of nutrition (Symonds et al., 2007; Lemley et al., 2014), and nutrient content of the amnion has been altered by nutrient restriction in ewes (Kwon et al., 2004), indicating that maternal nutrition may have an even greater impact during final prenatal maturation. Small intestinal function is particularly important in livestock species that rely upon transfer of passive immunity from immunoglobulins in colostrum (e.g. cattle and sheep). Colostrum also contains a cadre of growth factors, hormones, and nutrients which are crucial for small intestinal development (Quigley et al., 1988; Xu, 1996; Sangild et al., 2000; Berni Canani et al., 2008). Colostrum production has been decreased by both nutrient restriction and over nutrition in ewes (Swanson et al., 2008; Meyer et al., 2011), which could also have further implications in perinatal small intestinal maturation.

There are few data from ruminant developmental programming models investigating small intestinal parameters postnatally. Two studies have investigated postnatal lamb small intestinal growth and vascularity after mid- and late gestation nutrient restriction or over-nourishment (**Table 2**). These data demonstrate that 20-d old lambs have continued alterations in jejunal hyperplasia, vascularity, and gene expression, even when lambs were fed a common artificial colostrum and milk replacer after birth and managed together. Moreover, jejunal proliferation, vascularity, and gene expression were also affected by gestational nutrition in 180-d old lambs in a similar model, demonstrating that changes to the small intestine may persist well into life. In both 20- and 180-d old lambs, glucagon-like peptide 2 (**GLP-2**) expression was altered, although in opposite ways (**Table 2**). This GLP-2 is very important for small intestinal development, including growth and vascularization, making it a possible mechanism for small intestinal changes observed in these studies.

It has also been demonstrated that maternal intake of specific nutrients such as selenium during gestation can impact fetal small intestinal development. Fetuses from ewes fed supranutritional selenium throughout gestation had increased jejunal hypertrophy and decreased jejunal VEGF mRNA expression. In addition, form and level of maternal selenium supplementation during gestation have impacted fetal jejunal hypertrophy. Even when lambs were fed similar diets postnatally, high selenium during gestation has continued to impact lamb jejunal measures at d 20 and 180 of age, suggesting long-term impacts of this micronutrient fed prenatally or compensation by offspring after normal selenium intakes postnatally.

Maternal Small Intestinal Adaptations

Adaptation to Nutrient Manipulation

Nutritional Plane. Small intestinal growth and function are known to change with nutrient intake, so it should come as no surprise that they change with nutritional plane during pregnancy. Most of the studies cited here include treatments that vary in nutrient intake and bulk density of feed, both of which impact the small intestine. These studies investigating impacts of nutritional plane during gestation on ruminant small intestinal mass, proliferation, vascularity, and gene expression are summarized in **Table 3**.

In general, alteration of nutritional plane during early gestation alone does not seem to affect mass of the ruminant small intestine (**Table 3**), even though over nutrition during this period increased indices of jejunal hypertrophy. Impacts of nutrient restriction during early and mid- or mid-gestation are more variable. These have either decreased or not affected maternal small intestinal mass when measured immediately after nutrient restriction. Dams rebounded when nutrient restriction was followed by realimentation in late gestation, and small intestinal mass was not different from controls near term.

In most studies, small intestinal mass has responded to nutritional plane during both mid- and late gestation or late gestation only when measured at the end of the restriction period (**Table 3**). Changes in cellularity have been observed in these studies indicating that both hypertrophy and hyperplasia may play a role in growth differences, even when no change in mass was observed. Despite differences in mass and cellularity, no differences have been observed in jejunal crypt cell proliferation due to nutritional plane. This is likely because tissues were collected from ewes after long periods (40 to 80 d) of nutrient restriction in these studies. Alterations in proliferative rate necessary to change small intestinal mass may have occurred much earlier during nutrient restriction, and the tissues most likely reached steady-state by late gestation. Small intestinal adaptation has been detected as soon as 5 to 14 d after dietary changes, supporting this hypothesis. Little is known about the impacts of gestational nutrition on small intestinal energy use, but one study reported that oxygen consumption was increased per unit of tissue in nutrient-restricted ewes. Jejunal vascularity has responded to nutritional plane during gestation in several studies in ewes (**Table 3**).

The mechanisms of adaptation to altered nutritional plane during gestation in both growth and vascularity of the ruminant small intestine are not well known, but angiogenic and vasoactive factor gene expression may play a role. Expression of VEGF and NO systems have been altered in ewes (**Table 3**), although some of these data are contradictory. Jejunal mRNA expression of VEGF and its receptors, FLT1 and KDR, were greater for nutrient-restricted ewes in late gestation, suggesting that up-regulation of angiogenic factors was occurring in the face of reduced small intestinal growth and vascularization. Jejunal expression of VEGF and endothelial NO synthase 3 (**NOS3**) have also been increased after over nutrition during pregnancy (Meyer et al, 2013). In vitro systems have demonstrated that VEGF delivery to the small intestine increases vascularity (Rocha et al., 2008), suggesting that the small intestine of both nutrient restricted and over-nourished ewes may use VEGF or its receptors to modulate vascularization during nutritional insults. It is important to point out that it is uncertain if angiogenic factors influenced vascularization changes earlier in the nutrient-restriction period, as gene expression was only determined at one time point.

Specific Nutrients. There have been few published studies to date investigating the effect of specific nutrient intake during gestation on the maternal small intestine. In a series of studies to determine impacts of supranutritional selenium in ewes during gestation, results have been variable. High selenium diets fed during gestation have had no effect (Neville et al., 2008; Carlson et al., 2009), increased (Reed et al., 2007), and decreased (Meyer et al., 2012) primiparous ewe small intestinal mass. When small intestinal mass was increased, no effects of selenium on cellularity measures, proliferation, or vascularity were observed (Reed et al., 2007). Alternatively, supranutritional selenium decreased DNA concentration in other studies (Neville et al., 2008; Carlson et al., 2009), with proliferative rate of crypt cells unaffected (Carlson et al., 2009) or increased by selenium (Neville et al., 2008). Expression of the VEGF and NO systems has been impacted by high selenium, where supranutritional selenium has reduced mRNA of VEGF and its receptors (Neville et al., 2010; Meyer et al., 2012). Selenium has been hypothesized to decrease cancerous tumor growth and vascularization (Zeng and Combes, 2008), thus actions of selenium on proliferation and vascularity of the small intestine may have similar mechanisms. When high selenium was removed from the diet during lactation, small intestinal mass of ewes increased within the first 20 d to that of control-fed ewes (Meyer et al., 2012). It is unclear what caused differences in responses to high selenium in these studies, although selenium source and level of supplementation appear to alter small intestinal response (Neville et al., 2008; 2010), and thus likely influenced results.

Future Directions

The small intestine is a dynamic, rapidly changing tissue that is crucial for animal growth and health. Further research is necessary to better understand the role of the maternal small intestine in providing nutrients to the fetus and postnatal offspring and to advance knowledge of the effects of maternal nutrition on programming of offspring small intestinal growth and function. Additionally, research in the role of epigenetics and the microbiome in programming of the small intestine is in its infancy and can provide a

wealth of knowledge. A better understanding of the effects of gestational nutrition on the maternal and offspring small intestine will allow for development of management and therapeutic strategies to optimize the efficiency of livestock production.

References

Avila, C. G., R. Harding, S. Rees, and P. M. Robinson. 1989. Small intestinal development in growth-retarded fetal sheep. J. Pediatr. Gastroenterol. Nutr. 8:507-515.

Berni Canani, R., A. Passariello, V. Buccigrossi, G. Terrin, and A. Guarino. 2008. The nutritional modulation of the evolving intestine. J. Clin. Gastroenterol. 42:S197-S200.

Carlson, D. B., J. J. Reed, P. P. Borowicz, J. B. Taylor, L. P. Reynolds, T. L. Neville, D. A. Redmer, K. A. Vonnahme, and J. S. Caton. 2009. Effects of dietary selenium supply and timing of nutrient restriction during gestation on maternal growth and body composition of pregnant adolescent ewes. J. Anim. Sci. 87: 669-680.

Caton, J. S., J. J. Reed, R. P. Aitken, J. S. Milne, P. P. Borowicz, L. P. Reynolds, D. A. Redmer, and J. M. Wallace. 2009. Effects of maternal nutrition and stage of gestation on body weight, visceral organ mass, and on indices of jejunal cellularity, proliferation, and vascularity in pregnant ewe lambs. J. Anim. Sci. 87: 222-235.

Cellini, C., J. Xu, A. Arriaga, and T. Buchmiller-Crair. 2004. Effect of epidermal growth factor infusion on fetal rabbit intrauterine growth retardation and small intestinal development. J. Pediatr. Surg. 39:891-897.

D'Inca, R, M. Kloareg, C. Gras-Le Guen, and I. Le Hueron-Luron. 2010. Intrauterine growth restriction modifies the developmental pattern of intestinal structure transcriptomic profile, and bacterial colonization in neonatal pigs. J. Nutr. doi: 10.3945/jn.109.116822.

Dube, P. E., and P. L. Brubaker. 2007. Frontiers in glucagon-like peptide-2: multiple actions, multiple mediators. Am. J. Physiol. 293:E460-E465.

Hales, C. N., and D. J. P. Barker. 1992. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. Diabetologia. 35:595-601.

Holmes, K, S. D. Charnock Jones, A. J. Forhead, D. A. Giussani, A. L. Fowden, D. Licence, S. Kempster, and G. C. S. Smith. 2008. Localization and control of expression of VEGF-A and the VEGFR-2 receptor in fetal sheep intestines. Ped. Res. 63:143-148.

Kwon, H, S. P. Ford, F. W. Bazer, T. E. Spencer, P. W. Nathanielsz, M. J. Nijlan, B. W. Hess, and G. Wu. 2004. Maternal nutrient restriction reduces concentrations of amino acids and polyamines in ovine maternal and fetal plasma and fetal fluids. Biol. Reprod. 71:901-908.

Lemley, C. O., A. M. Meyer, T. L. Neville, D. M. Hallford, L. E. Camacho, K. R. Maddock-Carlin, T. A. Wilmoth, M. E. Wilson, G. A. Perry, D. A. Redmer, L. P. Reynolds, J. S. Caton, and K. A. Vonnahme. 2014. Dietary selenium and nutritional plane alters maternal endocrine profiles during pregnancy and lactation. Domestic Animal Endocrinology. Domestic Animal Endocrinology 46:1-11.

Meyer, A. M., B. W. Hess, S. I. Paisley, M. Du, and J. S. Caton. 2014. Small intestinal growth measures are correlated with feed efficiency in market weight cattle, despite alteration of maternal nutrition during gestation. J. Anim. Sci. 92:3855-3867.

Meyer, A. M., T. L. Neville, J. J. Reed, J. B. Taylor, L. P. Reynolds, D. A. Redmer, C. J. Hammer, K. A. Vonnahme, and J. S. Caton. 2013. Maternal nutritional plane and selenium supply during gestation impacts visceral organ mass and intestinal growth and vascularity of neonatal lamb offspring. J. Anim. Sci. 91:2628-2639.

Meyer, A. M., J. J. Reed, T. L. Neville, J. B. Taylor, C. J. Hammer, L. P. Reynolds, D. A. Redmer, K. A. Vonnahme, and J. S. Caton. 2010. Effects of nutritional plane and selenium supply during gestation on ewe and neonatal offspring performance, body composition, and serum selenium. J. Anim. Sci. 88:1786-1800.

Meyer, A. M., J. J. Reed, T. L. Neville, J. F. Thorson, K. R. Maddock-Carlin, J. B. Taylor, L. P. Reynolds, D. A. Redmer, J. S. Luther, C. J. Hammer, K. A. Vonnahme, and J. S. Caton. 2011. Nutritional plane and selenium supply during gestation impact on yield and nutrient composition of colostrum and milk in primiparous ewes. J. Anim. Sci. 89:1627-1639.

Meyer, A. M., J. J. Reed, T. L. Neville, J. B. Taylor, L. P. Reynolds, D. A. Redmer, K. A. Vonnahme, and J. S. Caton. 2012. Effects of nutritional plane and selenium supply during gestation on visceral organ mass and indices of intestinal growth and vascularity in primiparous ewes at parturition and during early lactation. J. Anim. Sci. 90:2733-2749.

Meyer, A. M., J. J. Reed, K. A. Vonnahme, S. A. Soto-Navarro, L. P. Reynolds, S. P. Ford, B. W. Hess, and J. S. Caton. 2010. Effects of stage of gestation and nutrient restriction during early to mid-gestation on maternal and fetal visceral organ mass and indices of jejunal growth and vascularity in beef cows. J. Anim. Sci. 88:2410-2424.

Neville, T. L., D. A. Redmer, , P. P. Borowicz, J. J. Reed, M. A. Ward, M. L. Johnson, J. B. Taylor, S. A. Soto-Navarro, K. A. Vonnahme, L. P. Reynolds, and J. S. Caton. 2010. Maternal dietary restriction and selenium supply alters mRNA expression of angiogenic factors in maternal intestine, mammary gland, and fetal jejunal tissues during late gestation in pregnant ewe lambs. J. Anim. Sci. 88: 2692-2702

Neville, T. L., M. A. Ward, J. J. Reed, S. A. Soto-Navarro, S. L. Julius, P. P. Borowicz, J. B. Taylor, D. A. Redmer, L. P. Reynolds, and J. S. Caton. 2008. Effects of level and source of dietary selenium on maternal and fetal body weight, visceral organ mass, cellularity estimates, and jejunal vascularity in pregnant ewe lambs. J. Anim. Sci. 86:890-901.

Prezotto, L. D., L. E. Camacho, C. O. Lemley, F. E. Doscher, J. S. Caton, K. A. Vonnahme, and K. C. Swanson. 2014. Effects of nutrient restriction and melatonin supplementation on maternal and foetal hepatic and small intestinal energy utilization. J Anim Physiol Anim Nutr (Berl). 98:797-807.

Qiu, X. S., T. T. Huang, Z. Y. Shen, H. Y. Deng, and Z Y. Ke. 2005. Effect of early nutrition on intestine development of intrauterine growth retardation in rats and its correlation to leptin. World Journal of Gastroenterology. 11:4419-4422.

Quigley, J. D., III., and J. J. Drewry. 1988. Nutrient and immunity transfer from cow to calf pre- and postcalving. J. Dairy Sci. 81:2779-2790.

Reed, J. J., M. A. Ward, K. A. Vonnahme, T. L. Neville, S. L. Julius, P. P. Borowicz, J. B. Taylor, D. A. Redmer, A. T. Grazul-Bilska, L. P. Reynolds, and J. S. Caton. 2007. Effects of selenium supply and dietary restriction on maternal and fetal body weight,

visceral organ mass, cellularity estimates, and jejeunal vascularity in pregnant ewe lambs. J. Anim. Sci. 85:2721-2733
Rocha, F. G., C. A. Sundback, N. J. Krebs, J. K. Leach, D. J. Mooney, S. W. Ashley, J. P. Vacanti, and E. E. Whang. 2008. The effect of sustained delivery of vascular endothelial growth factor on angiogenesis in tissue-engineered intestine. Biomaterials. 29:2884-2890.
Rompala, R. E., T. A. Hoagland, and J. A. Meister. 1988. Effect of dietary bulk on organ mass, fasting heat production and metabolism of the small and large intestine in sheep. J. Nutr. 118:1553-1557.
Sangild, P. T., A. L. Fowden, and J. F. Trahair. 2000. How does the foetal gastrointestinal tract develop in preparation for enteral nutrition after birth. Livest. Prod. Sci. 66:141-150.
Scheaffer, A. N., J. S. Caton, D. A. Redmer, D. R. Arnold, and L. P. Reynolds. 2004a. Effect of dietary restriction, pregnancy, and fetal type on intestinal cellularity and vascularity in Columbia and Romanov ewes. J. Anim. Sci. 82:3024-3033.
Scheaffer, A. N., J. S. Caton, D. A. Redmer, and L. P. Reynolds. 2004b. The effect of dietary restriction, pregnancy, and fetal type in different ewe types on fetal weight, maternal body weight, and organ mass in mature ewes. J. Anim. Sci. 82:1826-1838.
Swanson, T. J., C. J. Hammer, J. S. Luther, D. B. Carlson, J. B. Taylor, D. A. Redmer, T. L. Neville, J. J. Reed, L. P. Reynolds, J. S. Caton, and K. A. Vonnahme. 2008. Effects of gestational plane of nutrition and selenium supplementation on mammary development and colostrum quality in pregnant ewe lambs. J. Anim. Sci. 86:2415-2423.
Symonds, M. E., T. Stephenson, D. S. Gardner, and H. Budge. 2007. Long-term effects of nutritional programming of the embryo and fetus: mechanisms and critical windows. Repro. Fertil. Dev.19:53-62.
Trahair, J. F., and P. T. Sangild. 2002. Studying the development of the small intestine: philosophical and anatomical perspectives. Edtion ed. In: R. Zabielski, P. C. Gregory, and B. Westrom, eds. Biol. of the Intest. Growing Anim. Amsterdam: Elsevier Science:1-54.
Trahair, J. F., T. M. DeBarro, J. S. Robinson, and J. A. Owens. 1997. Restriction of nutrition in utero selectively inhibits gastrointestinal growth in fetal sheep. J. Nutr. 127:637-641.
Wang, J, L. Chen, D. Li, Y. Yin, X. Wang, P. Li, L. J. Dangott, W. Hu, and G. Wu. 2008. Intrauterine growth restriction affects the proteomes of the small intestine, liver, and skeletal muscle in newborn pigs. J. Nutr. 138:60-66.
Wang, T, Y. J. Huo, F. Shi, R. J. Xu, and R. J. Hutz. 2005 Effects of intrauterine growth retardation on development of the gastrointestinal tract in neonatal pigs. Biol. Neonate. 88:66-72.
Wells, J. C. K. 2007.The thrifty phenotype as an adaptive maternal effect. Biol. Rev. 82:143-172.
Xu, R. J. 1996. Development of the newborn GI tract and its relation to colostrum/milk intake: A review. Repro. Fertil. Dev. 8:35-48.
Yunusova, R., T. L. Neville, K. A. Vonnahme, C. J. Hammer, J. J. Reed, J. B. Taylor, D. A. Redmer, L. P. Reynolds, and J. S. Caton. 2013. Impacts of maternal selenium

supply and nutritional plane on visceral tissues and intestinal biology in offspring. J. Anim. Sci. 91:2229-2242.
Zeng, H., and G. F. Combs, Jr. 2008. Selenium as an anticancer nutrient: roles in cell proliferation and tumor cell invasions. J. Nutr. Biochem.19:1-7.

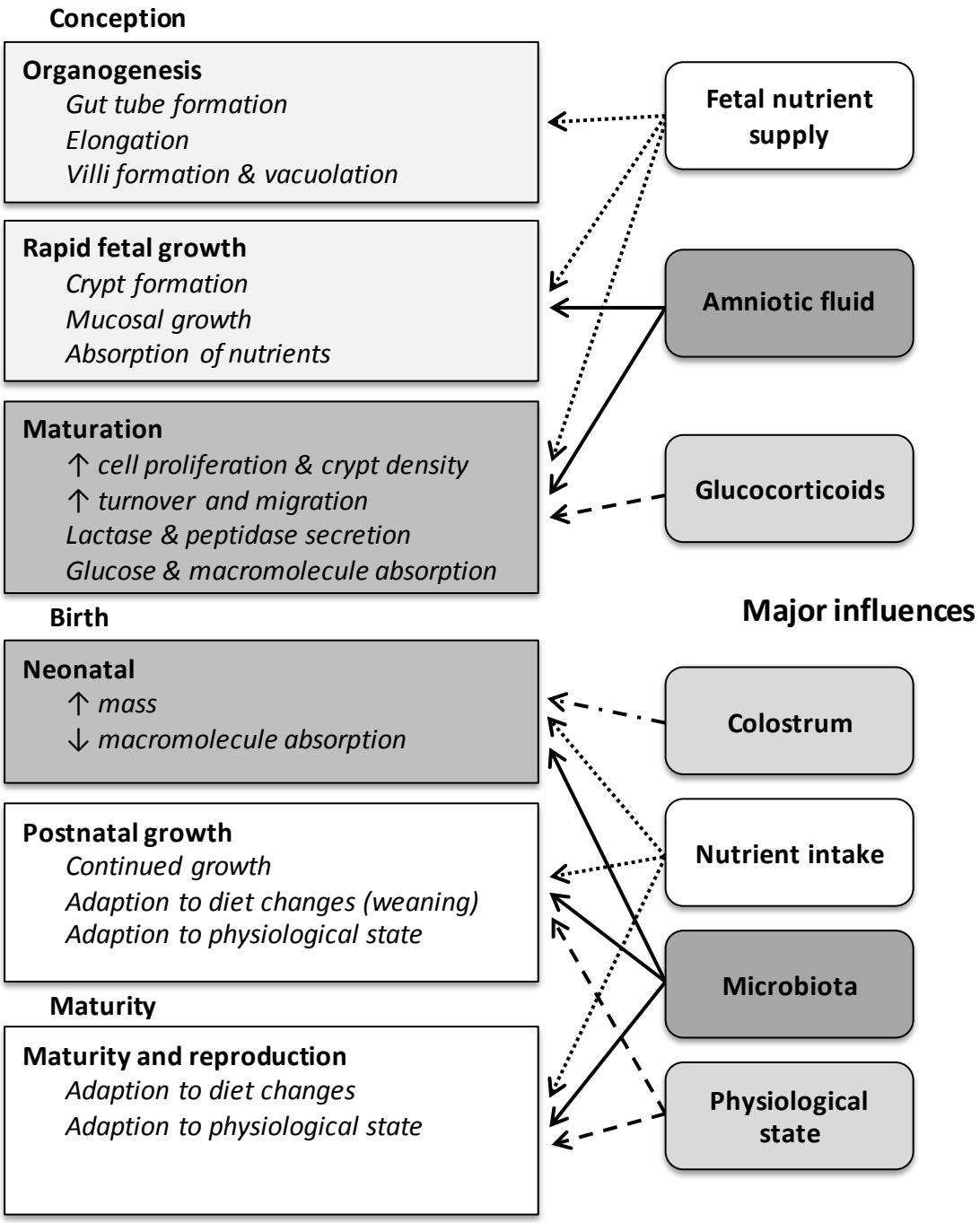


Figure 1. Windows of small intestinal growth and development and their influences. The timing of these events vary with species, but in general organogenesis occurs during early to mid-gestation, rapid fetal growth occurs in mid- to late gestation, and maturation occurs during late gestation, immediately before birth. Adapted from Adv. Nut. 2016. 7:169–178.

Table 1. Impacts of intrauterine growth restriction on the small intestine (Adapted from Adv, Nutr. 2016. 7:169–178).[1]

Reference	Species	Age measured[2]	Small intestinal mass or length response	Additional small intestinal responses
Avila et al., 1989	Sheep	d 140 gestation	↓ mass ↓ length	↓ villus and crypt density ↓ villus height and crypt depth ↓ mucosal thickness
Trahair et al., 1997	Sheep	d 90 gestation	↓ mass ↓ relative mass	↓ mucosal circumference and area ↓ crypt depth ↓ or abnormal enterocyte differentiation
Cellini et al., 2004	Rabbits	d 31 gestation	Not determined	↓ villus height ↓ proliferation ↑ epidermal growth factor mRNA
Qiu et al., 2005	Rats	birth to 12 wk	↓ mass (to 4 wk) ↓ length (to 12 wk)	↑ maltase (at birth) ↑ lactase (at birth)
Wang et al., 2005	Pigs	birth	↓ mass ↓ length	↓ mucosal weight ↓ IGF-1 mRNA expression
Wang et al., 2008	Pigs	birth	↓ mass ↓ relative mass	altered proteome
D'Inca et al., 2010	Pigs	birth to 5 d	↓ mass (to 2 d) ↓ length (to 5 d)	↓ villus height (to 2 d) ↓ villus width (at 2 d) ↑ adherent bacterial number altered transcriptome

[1] IGF-1, insulin-like growth factor 1.
[2] Approximate gestation lengths: sheep = 150 d, rabbit = 31 d.

Table 2. Impacts of maternal nutrition on the ruminant offspring small intestine from selected studies (Adapted from Adv. Nutr. 2016. 7:169–178)[1].

Reference	Species	Treatments	Age measured[2]	Small intestinal mass response	Additional small intestinal responses
Meyer et al., 2010	Cattle	CON vs RES (d 30 to 125 of gestation)	d 125 gestation	NS	↑ proliferation in RES
Meyer et al., 2010	Cattle	CON vs RES (d 30 to 125) and realimented (d 125 to 245)	d 245 gestation	NS	↑ total vascularity in RES and realimented
Meyer et al., 2014	Cattle	CON vs RES vs RES + AA supplement (d 45 to 185 gestation)	~450 d postnatal	NS	↑ GUCY1B3 mRNA in RES + AA
Prezotto et al., 2014	Sheep	CON vs RES (d 50 to 130 of gestation)	d 130 gestation	NS	↓ protein concentration in RES ↑ oxygen consumption in RES
Reed et al., 2007; Neville et al., 2010	Sheep	CON vs RES (d 64 to 135 of gestation)	d 135 gestation	↓ in RES	↓ total vascularity in RES ↓ protein:DNA in RES ↓ GUCY1B3 mRNA in RES

Meyer et al., 2010; 2013	Sheep	CON vs RES (d 40 of gestation to birth)	d 20 postnatal	NS	↓ total vascularity in RES ↓ capillary surface density in RES ↑ capillary size in RES ↑ GLP-2 mRNA in RES ↓ postnatal weight gain in RES
Yunusova et al. (55)	Sheep	CON vs RES (d 50 gestation to birth)	d 180 postnatal	NS	↓ capillary size in RES ↓ total proliferation in RES ↓ GLP-2 mRNA in RES
Meyer et al. (85, 86)	Sheep	CON vs OVR (d 40 of gestation to birth)	d 20 postnatal	NS	↑ DNA concentration in OVR
Yunusova et al., 2013	Sheep	CON vs OVR (d 50 of gestation to birth)	d 180 postnatal	NS	↓ total proliferating cells in OVR

[1] CON: control nutritional plane (near nutrient requirements); GLP-2: glucagon-like peptide 2; GUCY1B3: soluble guanylate cyclase (NO receptor); NS: not significant ($P > 0.10$); OVR: over nutrition; RES: nutrient restriction; RES + AA: nutrient restriction with protein supplementation to meet essential AA of control.
[2] Approximate gestation lengths: cattle = 285 d, sheep = 150 d.

Table 3. Impacts of gestational nutrition on maternal small intestine from selected studies (Adv. Nutr. 2016. 7:169–178)[1].

Reference	Species, parity	Treatments	Stage measured[2]	Small intestinal mass response	Additional small intestinal responses
Meyer et al., 2010	Cattle, Multiparous	CON vs RES (d 30 to 125 gestation)	d 125 gestation	NS	↓ RNA:DNA in RES
Meyer et al., 2010	Cattle, Multiparous	CON vs RES (d 30 to 125) and realimented (d 125 to 245)	d 245 gestation	NS	↓ RNA:DNA in RES
Scheaffer et al., 2004a,b	Sheep, Multiparous	CON vs RES (d 50 to 90)	d 90 gestation	↓ in RES	↓ DNA concentration in RES ↑ capillary area density in RES
Carlson et al., 2009	Sheep, First	CON vs RES (d 50 to 90)	d 130 gestation	NS	NS
Carlson et al., 2009	Sheep, First	CON vs RES (d 50 to 130)	d 130 gestation	↓ in RES	↓ DNA concentration in RES
Scheaffer et al., 2004a,b	Sheep, Multiparous	CON vs RES (d 50 to 130)	d 130 gestation	↓ in RES	↑ DNA concentration in RES ↑ capillary area density in RES
Prezotto et al., 2014	Sheep, First	CON vs RES (d 50 to 130)	d 130 gestation	↓ in RES	↑ oxygen consumption in RES
Carlson et al., 2009	Sheep, First	CON vs RES (d 90 to 130)	d 130 gestation	↓ in RES	↑ RNA concentration in RES

Reed et al., 2007; Neville et al., 2010	Sheep, First	CON vs RES (d 64 to 135)	d 135 gestation	↓ in RES	↓ total vascularity in RES ↓ capillary area density in RES ↓ capillary size in RES ↑ VEGF, FLT1, KDR mRNA in RES ↑ NRP1, NRP2 mRNA in RES
Meyer et al., 2012	Sheep, First	CON vs RES (d 40 to parturition)	d 0 post-partum	NS	↓ RNA concentration and RNA:DNA in RES ↓ capillary surface density in RES ↓ mucosal density in RES
Meyer et al., 2012	Sheep, First	CON vs RES (d 40 to parturition)	d 20 post-partum	NS	↑ proliferation in RES ↓ capillary surface density in RES
Caton et al., 2009	Sheep, First	CON vs OVR (d 0 to 50)	d 50 gestation	NS	↑ RNA concentration and RNA:DNA in OVR
Caton et al., 2009	Sheep, First	CON vs OVR (d 0 to 90)	d 90 gestation	↑ in OVR	↑ RNA concentration and RNA:DNA in OVR
Caton et al., 2009	Sheep, First	CON vs OVR (d 0 to 130)	d 130 gestation	↓ in OVR	↑ RNA concentration in OVR
Meyer et al., 2012	Sheep, First	CON vs OVR (d 40 to parturition)	d 0 post-partum	↑ in OVR	↓ RNA concentration and RNA:DNA in OVR ↑ total vascularity in OVR ↑ VEGF, FLT1 mRNA in OVR ↑ NOS3 mRNA in OVR
Meyer et al., 2012	Sheep, First	CON vs OVR (40d to parturition)	d 20 post-partum	NS	↓ proliferation in OVR ↑ total vascularity in OVR

[1] CON: control nutritional plane; FLT1: VEGF receptor 1; KDR: VEGF receptor 2; NOS3: endothelial nitric oxide synthase 3; NRP1: neuropilin 1; NRP2: neuropilin 2; NS: not significant ($P > 0.05$); OVR: over nutrition; RES: nutrient restriction; VEGF: vascular endothelial growth factor.

[2] Approximate gestation lengths: cattle = 285 d, sheep = 150 d.

Can We Modify Future Beef Calf Performance by Changing Cow Nutrition During Gestation?

Philipe Moriel[1]
Range Cattle Research & Education Center – University of Florida

Introduction

The beef cattle industry in the southeastern US relies primarily on the use of high-forage diets to develop replacement heifers, maintain the cow herd, and sustain 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 nutritional 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 situations and a lack of forage frequently observed throughout the state.

There are two typical priorities related to feeding beef cows. First, provide the cheapest diet possible to reduce annual feeding costs and secondly, provide enough nutrients to prevent reproductive failure. It is well known that poor cow nutrition can decrease reproductive performance. If cows' nutrient requirements are not met before calving, they will start mobilizing nutrients from their own reserves to survive and to maintain fetal calf growth. Consequently, it is likely that these cows will calve at a low body condition score **(BCS**). The BCS system is an indicator of the percentage of body fat during the cow's production cycle, and it is a crucial determinant of their reproductive performance and productivity. Cows will not conceive at an acceptable rate (generally >85%) without adequate body fat reserves (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 as shown in **Figure 1**. As BCS at calving decreases, pregnancy rates also decrease (**Figure 1**). 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 cow nutrition on subsequent calf growth and health (fetal programming concept).

[1] Contact: 3401 Experiment Station, Ona, FL 33865, Telephone: (863) 735-1314 ext. 208, E-Mail: pmoriel@ufl.edu

Fetal programming

Fetal programming is the concept that a maternal stimulus or insult at a critical period in fetal development has long-term effects on the offspring (Funston et al., 2010). Approximately 75% of calf fetus 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 of calf 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 develop during early gestation, whereas muscle and adipose tissue formation occurs primarily during mid to late gestation (Du et al., 2010). Thus, nutrient restriction during gestation might impact placental formation and calf organ development. Also, depending on when the nutrient restriction happens during gestation, the outcome of this insult might have different consequences on calf performance. We will report how cow nutrient restriction during early, mid, and late gestation might differently affect the subsequent calf performance.

Consequences of Nutrient Restriction

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, 1996), especially if cows are managed to calve during the dry or winter seasons when poor forage quality and quantity is available.

Unfortunately, a limited amount of published results exists regarding 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 the 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. Understandably, 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 on calf birth weight, weaning weights, and average daily gain from birth to weaning or during the

feedlot finishing phase (**Table 1**). However, lung and trachea weights of steers born to heifers provided 55% of their nutrient requirements were significantly less than steers born to heifers 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.

Mid Gestation (3 to 6 months of gestation)

Production-oriented tissues, such as muscle, appear to be responsive to fetal programming effects in utero (Caton and Hess, 2010). Muscle formation is divided into two waves of muscle fiber synthesis. The first wave begins at mid gestation, whereas the second wave occurs from six to nine months of gestation (Du et al., 2010). Thus, 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 grazed on low-quality, native pastures (6% crude protein) or high-quality, fertilized and irrigated pastures (11% crude protein) for 60 days from 120 to 150 days through 180 to 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 grazed on native pastures compared to male offspring born to cows grazed on improved pastures during mid gestation (**Table 2**). In addition, the Warner-Bratzler shear force, which is an 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 other words, 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 also may have consequences on organ development. Angus × Gelbvieh cows were randomly allotted into groups and fed at 70 or 100% of their nutrient requirements from day 45 to 185 of gestation. They were then commingled and fed at 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.

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. As mentioned before, major portions of beef cattle muscle and adipose tissue form during late gestation (Du et al., 2010). Muscle fiber number is set at birth, meaning that after the calf is born, 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 accumulating fatty acids and generating intramuscular fat, for example), resulting in carcasses with lower quality and marbling scores.

In a series of studies from the University of Nebraska (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 3**). Cows were sorted into groups that received or did not receive 1 lb/day of a protein supplement (42% crude protein) during late gestation. All studies reported that male offspring born to cows that received the protein supplement were heavier than male offspring born to non-supplemented cows. In addition, two of those three 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 born from cows that were supplemented with protein during late gestation.

Similar studies from the University of Nebraska also evaluated the effects of supplementing beef cows with 1 lb/day of a protein supplement during late gestation (**Table 4**). 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 during late gestation. In addition, heifers born to cows that were supplemented achieved puberty at younger ages (Funston et al., 2010) and had greater pregnancy rates (Martin et al., 2007) than heifers born to cows that did not receive protein supplementation (**Table 4**).

Progeny 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 heifers receiving 65% of their dietary energy requirement over the last 90 days of gestation compared with calves from primiparous heifers receiving 100% of their energy requirement. A potential factor contributing to increased morbidity and mortality is decreased calf birth weight. Calves born to nutrient-restricted cows were 5 lb 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 1 lb/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 to cows that were fed 70% of energy requirements during the last 40 days of gestation had lower overall plasma concentrations of cortisol (indicator of stress level) and haptoglobin (indicator of inflammatory response) compared to calves born to cows fed at maintenance levels (**Table 5**). Also, calves born to cows that were energy restricted during late gestation produced less antibodies against bovine viral diarrhea virus, 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 and potentially "weaker" than calves born to cows that were fed at maintenance levels during late gestation. Therefore, even though calf growth performance was not affected, calves might be more susceptible to diseases if they are born to cows that were energy restricted. More studies need to be conducted in this research area as it has substantial implications to cow-calf producers, and this need will be addressed by our research group at Ona, FL.

Fetal-programming research in Florida Beef Herds

It is important to highlight that all 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 most pastures in FL. It is unknown if cows and calves will experience similar positive (or negative) results mentioned above under our environmental conditions. Thus, starting in May 2017, our research group will focus on evaluating the impact of fetal programming on growth, reproduction, health, and carcass quality of offspring born to cows grazing warm-season grasses and exposed to climatic conditions of FL.

To begin our efforts, we successfully obtained funding from the FL Cattle Enhancement Fund from FL Cattlemen's Association to conduct 2 long-term experiments at the Range Cattle Research & Education Center (Ona, FL) and commercial operations located in the South/Central part of FL.

Experiment 1 will begin in May 2017 and will evaluate if year-round supplementation of energy and protein could improve cow reproductive success and offspring performance following birth compared to a Fall/Winter supplementation program traditionally used in FL beef cattle operations. Pregnant cows will be sorted into 3 groups, and will be provided molasses supplementation from calving until the end of the breeding season (CONTROL), or year round supplementation of molasses or range cubes. Total annual amount of supplement will be similar among all treatments

(approximately 600 lb of supplement dry matter/cow annually). Optimal BCS at calving is one of the most important factors needed to obtain successful pregnancy rates. Cows supplemented year-round might achieve a greater BCS at calving without increasing the annual supplement amount. Another advantage is that the trace mineral salt can be mixed into the supplement, reducing annual fluctuations in voluntary intake and wastage of free-choice trace mineral formulations, and simultaneously improve cow trace mineral status. We believe that year-round supplementation of molasses or range cubes will increase BCS at calving and trace mineral status of cows throughout the year, which will enable cows to experience greater BCS loss during early-lactation without reducing their reproductive performance compared to cows supplemented with molasses during the Winter/Fall season only. In addition, year-round supplementation of molasses and range cubes will improve calf development during pregnancy, and then, improve calf health, survivability, and growth following birth.

Experiment 2 will begin in September 2017 and will evaluate: (1) if supplementation of Brangus cows during the entire late-gestation period (1 lb/day of protein supplement for 90 days = 90 lb per cow) will increase reproductive success of cows, calf development during gestation and performance after birth to levels higher than the cost of this supplementation strategy, and (2) if concentrating cow supplementation during their period of lowest nutrient demand (first 30 days after weaning) will be more cost-effective than cows supplemented during the entire late-gestation period. We believe 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. We also believe that supplementing 3 lb/day for 30 days after weaning will reduce feeding costs, have the greatest improvement on cow weight gain and reproduction success, but not cause fetal-programming effects (due to the shorter supplementation period). In contrast, supplementation of 1 lb/day for 90 days will have greater labor costs, lower improvement on reproduction, but enhance calf development during gestation and performance after birth.

Conclusions

Nutrient deficiency often occurs in animals provided forage-based diets due to seasonal variation in forage quality and quantity, and because of mismanagement leading to overgrazed pastures. This nutrient deficiency has been shown to impact the reproductive performance of cows, the subsequent growth and reproductive performance of calves, and meat quality. 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. Furthermore, this publication focused solely on the effects of gestational nutrient restriction. It is important to realize that excessive nutrient consumption (energy, protein, minerals, vitamins, and fatty acids), diet composition (starch concentration), energy and protein sources, and stress also have potential for programming calf development in utero. Thus, cow-calf nutrition termed “fetal programming” has large implications for the beef industry and merits producer attention and further research attention in the future.

References

Caton, J. S., and B. W. Hess. 2010. Maternal plane of nutrition: Impacts on fetal outcomes and postnatal offspring responses. In *Proc. Graz. Lives. Nut. Conf*., 104-119. Casper, WY.

Corah, L. R., T. G. Dunn, and C. C. Kaltenbach. 1975. Influence of prepartum nutrition on the reproductive performance of beef females and the performance of their progeny. J. Anim. Sci. 41:819–824.

Du, M., J. Tong, J. Zhao, K. R. Underwood, M. Zhu, S. P. Ford and P. W. Nathanielsz. 2010. Fetal programming of skeletal muscle development in ruminant animals. J. Anim. Sci. 88:E51-E60.

Funston, R. N., D. M. Larson, and K. A. Vonnahme. 2010. Effects of maternal nutrition on conceptus growth and offspring performance: Implication for beef cattle production. J. Anim. Sci. 88:E205-E215.

Funston, R. N., A. F. Summers, and A. J. Roberts. 2012. Implications of nutritional management for beef cow-calf systems. J. Anim. Sci. 90:2301-2307.

Hubbert, W. T., O. H. V. Stalheim, and G. D. Booth. 1972. Changes in organ weights and fluid volumes during growth of the bovine fetus. Growth 36:217-233.

Larson, D. M., J. L. Martin, D. C. Adams, and R. N. Funston. 2009. Winter grazing system and supplementation during late gestation influence performance of beef cows and steer progeny. J. Anim. Sci. 87:1147–1155.

Long, N. M., M. J. Prado-Cooper, C. R. Krehbiel, U. DeSilva, and R. P. Wettemann. 2010. Effects of nutrient restriction of bovine dams during early gestation on postnatal growth, carcass and organ compositions, and gene expression in adipose tissue and muscle. J. Anim. Sci. 88:3251–3261.

Long, N. M., C. B. Tousley, K. R. Underwood, S. I. Paisley, W. J. Means, B. W. Hess, M. Du and S. P. Ford. 2012. Effects of early- to mMid-gestational undernutrition with or without protein supplementation on offspring, growth, carcass characteristics, and adipocyte size in beef cattle. J. Anim. Sci. 90:197-206.

Martin, J. L., K. A. Vonnahme, D. C. Adams, G. P. Lardy, and R. N. Funston. 2007. Effects of dam nutrition on growth and reproductive performance of heifer calves. J. Anim. Sci. 85:841–847.

Moriel, P., L. F. A. Artioli, M. B. Piccolo, M. H. Poore, R. S. Marques, and R. F. Cooke. 2016. Short-term energy restriction during late gestation and subsequent effects on postnatal growth performance, and innate and humoral immune responses of beef calves. J. Anim. Sci. 94:2542-2552.

NRC. 1996. Nutrient Requirements of Beef Cattle. 7th rev. ed. Washington, DC: Natl. Acad. Press.

Robinson, J. J., I. McDonald, C. Fraser, and I. McHattie. 1977. Studies on reproduction in prolific ewes. I. Growth of the products of conception. J. Agric. Camb. 88:539-552.

Selk, G. E., R. P. Wettemann, K. S. Lusby, J. W. Oltjen, S. L. Mobley, R. J. Rasby and J. C. Garmendia. 1988. Relationships among weight change, body condition and reproductive performance of range beef cows. J. Anim. Sci. 66:3153-3159.

Stalker, L. A., D. C. Adams, T. J. Klopfenstein, D. M. Feuz, and R. N. Funston. 2006. Effects of pre- and postpartum nutrition on reproduction in spring calving cows and calf feedlot performance. J. Anim. Sci. 84:2582–2589.
Stalker, L. A., L. A. Ciminski, D. C. Adams, T. J. Klopfenstein, and R. T. Clark. 2007. Effects of weaning date and prepartum protein supplementation on cow performance and calf growth. Rangeland Ecol. Manage. 60:578–587.
Underwood, K. R., J. F. Tong, P. L. Price, A. J. Roberts, E. E. Grings, B. W. Hess, W. J. Means, and M. Du. 2010. Nutrition during mid to late gestation affects growth, adipose tissue deposition, and tenderness in cross-bred beef steers. Meat Sci. 86:588–593.
Zhu, M. J., S. P. Ford, P. W. Nathanielsz, and M. Du. 2004. Effect of maternal nutrient restriction in sheep on the development of fetal skeletal muscle. Biol. Reprod. 71:1968–1973.

Figure 1. Pregnancy rates of cows calving at different body condition scores (BCS; Selk et al., 1988; n = 300 multiparous cows).

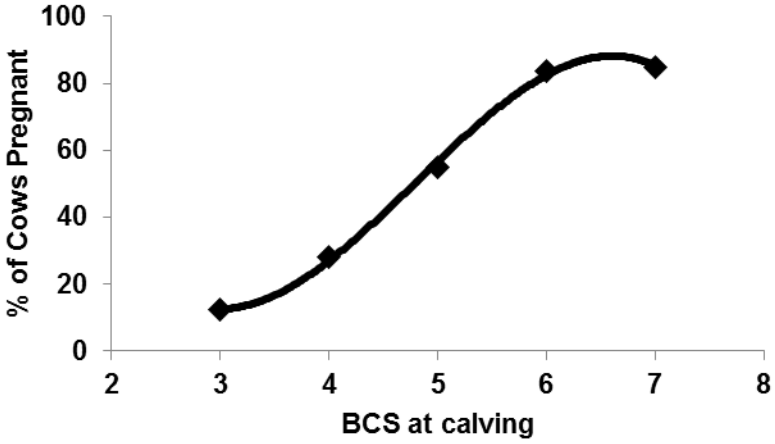


Figure 2. Lung plus trachea weights of steers born to first-calf heifers provided 55 or 100% of their nutrient requirements during the first 83 days of gestation (n = 10 steers per treatment; *$P < 0.05$).

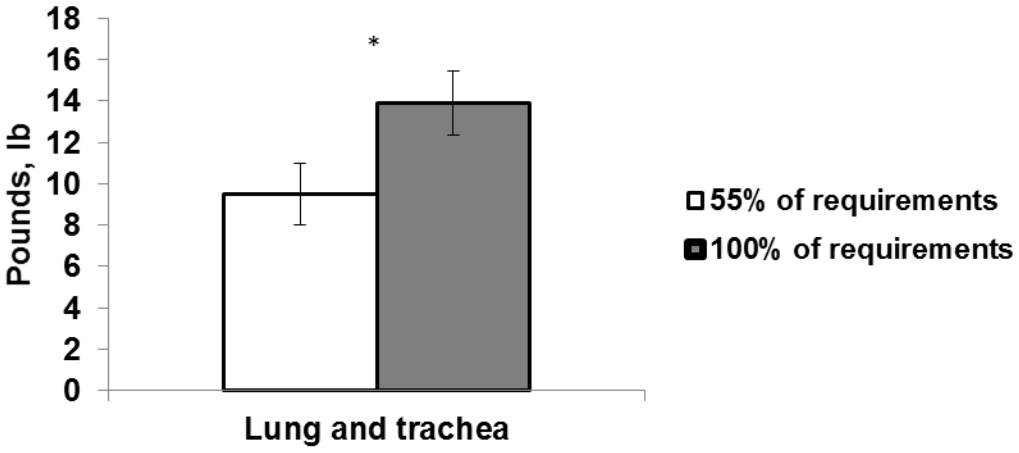


Figure 3. Wet ovary and luteal tissue weights of heifers born to cows provided 70 or 100% of their nutrient requirements from 45 to 185 days of gestation (Long et al., 2012; n = 4 heifers per treatment; 13 months of age; *P < 0.05).

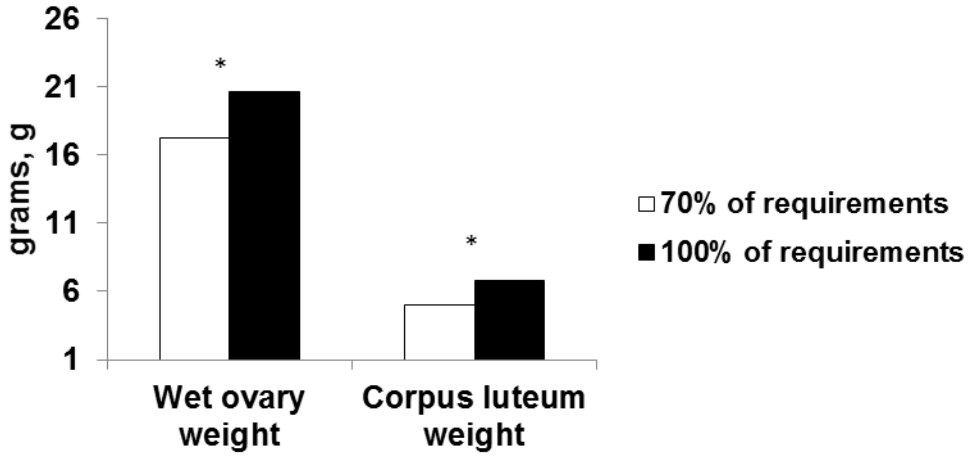


Table 1. Growth performance of male offspring born to first-calf heifers fed 55 or 100% of their nutrient requirements during the first 83 days of gestation (Long et al., 2010).

	Steers born to heifers fed:			
	55% of requirements	100% of requirements	SEM	*P*-value
Body weight, lb				
Birth	69	71	2.8	0.31
Weaning	491	480	26.4	0.32
Average daily gain, lb				
Birth to weaning	1.8	1.9	0.08	0.14
During finishing	4.9	4.6	0.28	0.40

Table 2. 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			
	Native pastures	Improved pastures	SEM	*P*-value
Birth, lb	85	81	4.4	0.46
At weaning, lb	533	564	8.1	0.02
At slaughter, lb	1145	1198	17.0	0.04
Hot carcass weight, lb	726	767	10.6	0.04

Table 3. Growth performance and carcass quality of male offspring born to cows that received (Supp.) or did not receive (No Supp.) protein supplementation (1 lb daily of a 42% crude protein supplement) during late gestation (*$P < 0.05$).

	Stalker et al. (2007)		Stalker et al. (2006)		Larson et al. (2009)	
Item	No Supp.	Supp.	No Supp.	Supp.	No Supp.	Supp.
Weaning weight, lb	441*	463*	465*	480*	518*	531*
Carcass weight, lb	764*	804*	800	813	802*	819*
Choice, %	-	-	85	96	71*	86*
Marbling	449	461	467	479	444*	493*

Table 4. Growth and reproductive performance of heifers born to cows that received (Supp.) or did not receive (No Supp.) protein supplementation (1 lb daily of a 42% crude protein supplement) during late gestation (*$P < 0.05$).

	Martin et al. (2007)		Funston et al. (2010)	
Item	No Supp.	Supp.	No Supp.	Supp.
Weaning weight, lb	456	467	496*	511*
Adj. 205-day weight	480*	498*	469	478
Age at puberty, days	334	339	366*	352*
Pregnancy rate, %	80*	93*	80	90

Table 5. 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
Post-weaning phase (day 266 to 306)				
ADG, lb	1.8	1.9	0.13	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
Serum antibody titers against				
BVD-1a, log_2	6.36	5.15	0.463	0.05