Nutrition and Immune Status of the Transition Cow; the Potential of Feed Additives

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SUMMARY

- Increased productivity in dairy cows has increased the challenges associated with the transition cow.
- Nutritional strategies to reducing the negative energy balance post-partum and increasing glucose supply will improve metabolic health and immune response; it should be part of the type of additive used during the transition period. Energy supply of the cow and the immune system should take an integrated approach.
- Propylene Glycol (PG) or glycerol should be a routine component of transition diets.
- Direct Feed Microbial (DFM) that stimulate rumen fermentation and improve energy supply are recommended as part of a transition diet.
- Rumen Protected Choline (RPC) is effective in avoiding fatty livers but needs to be evaluated in the presence of other methyl donors.
- Anti-oxidants notably Se and Vit. E are basic components of the transition cow diet.
- Complementarities and synergies among additives are important and need to be considered.

INTRODUCTION

Over the last decennia productivity and management of the dairy cow has changed dramatically. In the USA and many other countries production has increased with an average of more than 100 kg/year over a period of 50 years (Oltenacu and Broom, 2010). The current level of production has been paralleled by a significant increase in metabolic, locomotion (laminitis) and fertility problems. The dry period and the ensuing period around parturition have been recognized as being at the origin of many these problems. This has resulted in an increased attention to the dairy cow at the end of the dry period and the beginning of lactation now commonly referred to as the transition period. It is this period that has become, over the last 20 – 30 years, the focus of much research resulting in a significant increase in our understanding of the biochemical and molecular processes associated with the transition period. This in turn has led to important changes in the recommendations regarding management and nutrition of the periparturient cow focusing primarily on the supply and metabolism of energy. Nevertheless, our understanding of the underlying processes remains incomplete underlining in part the multi-faceted aspect of the transition cow problem and the many interactions between e.g. energy metabolism and other physiological processes.

This is especially true for the immune system whose importance during the transition period is now well accepted. The role of the immune system and the interactions between metabolism and immunity has more recently received increased attention (e.g. Sordillo, 2014; Waldron, 2007). The depressed immune system of the cow around calving, associated with the dramatic changes in circulating metabolites, is thought to be at the basis of the high disease incidence postpartum and the subsequent low performance.

In an effort to better regulate or attenuate the abrupt changes in nutrient supply and immune responses, there is an interest to look beyond the relatively short 6 weeks period that classically represents the transition period. Changing to a shorter dry period, reducing post-partum energy demands and fluxes in circulating metabolites may be a first step in offering practical solutions (Santchi and Lefebre, 2014; Grummer and Rastani, 2004; Shoshani et al. 2014). Coupled with the feeding of a ration formulated for a

more favorable ratio between glucogenic and lipogenic precursors, may improve metabolism and overall health status (van Knegsel et al., 2014).

Confronted with the need for practical solutions and, as a logical consequence of the large number of studies, a number of additives have been developed (and are being developed). Many of these additives have proven to be effective aids in avoiding metabolic problems or reducing their impact and have now become routine components of transition cow rations. This despite the fact that they often only address one particular aspect of the metabolic and/or immune challenges of the periparturient cow. Since the value of these additives is well recognized and management of the underlying causes remains complex "stacked solutions" (based on combinations of these additives) are offered and often included in the transition diet. These solutions aim at combining beneficial effects on energy metabolism with stimulatory action on the liver and immune system. A better understanding of these additives and the conditions under which they need to be applied, will allow for a more judicial selection and application.

ENERGY SUPPLY DURING THE TRANSITION-PERIOD; METABOLISM AND IMMUNE STATUS

At the end of the lactation period and much of the dry period cows are in a positive energy balance. At this stage the greatest risk in terms of energy supply is a relative excess resulting in over-conditioned cows. While the ideal body condition score for these cows is 3.25, even at moderate levels of dietary energy dry cows tend to consume more net energy (NE) than needed (Drakley and Janovick-Guretzky, 2007). This occurs despite the use of low energy diets generally rich in roughages and fiber. The first objective of these diets in the far-off dry cow is to control changes in body weight and BCS which generally equates to maintaining dry matter intake while limiting energy supply with the intention to correct the normal decrease in dry matter intake (DMI) of the dry cow. DMI in the weeks before calving is known to decrease (Bertics et al. 1992) and is thought to be a major factor in the post-calving changes in blood metabolites resulting from excessive tissue (esp. fat and Ca) mobilization. The pre-calving decline in DMI appears to be inversely correlated to BCS which predisposes over-conditioned cows to greater metabolic health problems.

Postpartum performance and well-being of the cow is improved by limiting the extent of negative energy balance. Strictly from an energy point of view, the objective of a successful transition cow feeding program is therefore to reduce fat mobilization and lower-blood levels of non-esterified fatty acids (NEFAs) and ketone bodies while increasing glucose and insulin. As part of this strategy feeding programs seek to maximize DM and energy intake after calving. Imposing a restricted energy supply during the dry period (below NRC 2001 recommendations) has shown to improve post calving intakes and reduce body fat mobilization as indicated by lower plasma NEFA levels (Douglas et al., 2006; Roche et al., 2005). Cows allowed to over-consume energy in the dry period have a lower energy balance, higher β − hydroxy butyrate (BHBA), liver triglycerides (TG) and NEFA levels at the onset of lactation (Dann et al., 2006). However, the literature is not unanimous; other studies have shown that in general, energy density of dry diets (from 28 d pre-calving to calving) only have a minor effect on post-partum metabolic status but that there are significant differences between primi- and multi-pari cows (Rabelo et al., 2005; Law et al 2011).

Lower energy intake during the dry period is often associated with lower plasma NEFAs and BHBA while insulin and glucose is not affected or only shows a small increase relative to cows fed higher energy diets (Dann et al., 2006; Douglas et al., 2006; Law et al 2011). This however, is often associated with lower milk production contributing to the reduction in negative energy balance. Restricted-fed animals appear to have an increased capacity for hepatic gluconeogenesis, β-oxidation and TAG accumulation in the liver (Roche et al., 2013) thus reducing the risk of metabolic problems which underlines the importance of controlling energy intake.

Similar metabolic profiles post-partum are obtained by reducing the dry period (Rastani et al., 2005; Remond et al., 1997; Shoshani et al. 2014) suggesting that a reduction in energy supply during the dry period will have a beneficial effect on disease incidence and herd health. It has been also been suggested (van Knegsel et al., 2014) that this can be further improved by feeding a more glucogenic diet (i.e. a diet that provides a larger proportion of glucogenic relative to lipogenic precursors, notably in the form of non-structural carbohydrates - at similar NE concentrations). Combined with the increased capacity for hepatic gluconeogenesis this type of diet improved liver health and the metabolic profile of the periparturient cow, notably glucose levels and increase insulin production (Chen et al., 2014). Indirectly, these results seem to be supported by the results obtained with feeding supplemental fat to increase energy supply and reduce NEFAs although the effects of specific, metabolically active fatty acids remains inconclusive (Overton and Waldron, 2004).

All cows experience some form of immune suppression around calving and it is now well accepted that the cow's immune status at this period plays a major role in managing metabolic problems. The period of reduced immunological capacity or immune dysfunction is not limited to isolated immune parameters but is rather broad in scope and affects various immune cell types (Waldron 2007). The differential effect of dry matter or energy intake on immune status during the dry period is difficult to discern. However, it is well recognized that nutrition and nutritional status (along with management factors) play a pivotal role in the immune response and that specific nutrients influence various aspects of the immune response. These aspects have recently been reviewed in a number of publications.

Glucose and ketones play a critical role in the effectiveness of the immune cells which is accentuated by the changes in metabolism during the transition phase. This is especially the case for glucose – already in short supply – whose requirement has been demonstrated for phagocytic cells. Glucose is preferred over other energy sources such as ketones or fatty acids by PMN, macrophages and lymphocytes. Consequently, it is to be expected that a reduction in circulating glucose - as is observed in periparturient cows - reduces their functionality (Ingvartsen and Moyes, 2013). Other energy substrates used by the immune cells are, at least in part, a direct result of the cow's metabolic status. The exact nature of the energetic demands and - how these are met - differs among immune cells and the type or level of response required. It is reasonable to assume that in the immune challenged periparturient cow, with reduced blood glucose levels; these energy substrates play an important role. This especially in light of the fact that specific fatty acids have direct regulatory actions on immune cells (i.e. leukocytes) (Wolowczuk et al 2008) and that these immune cells appear to be selective in which fatty acids to incorporate from the NEFA or blood phospholipid fraction (Contreras et al., 2010).

On the other hand, a number of studies exist that suggest a direct inhibitory effect of NEFAs and BHBA on specific immune cell populations (Ingvartsen and Moyes, 2013; Sordillo and Mavangira, 2014). From a strict immunological point of view, maximizing blood glucose supply and reducing NEFA and ketone levels should be beneficial.

TRANSITION COW ADDITIVES TO ENHANCE ENERGY METABOLISM AND IMMUNITY

Improving Energy and Glucose Supply

The use of energy supplements as glucogenic precursors in the form of oral drenches to prevent or treat ketosis is more than half a century old. Originally this concerned mainly PG and calcium propylene but more recently glycerol (also called glycerin) has been added to the list. Because of its effectiveness PG and glycerol are now commonly used in transition cow diet as part of a TMR mix or as top feeding. Due to the price differential the most widely used compound is feed grade glycerol. Results of in vitro and in vivo fermentation studies indicate that glycerol is rapidly fermented and will increase rumen propionate and butyrate (Remond, 1993). This also appears to be the case when PG is fed but PG's effect on propionate is larger than that of glycerol. Consequently, supplying PG or glycerol affects microbial fermentation with a significant increase of not only the glucose precursor propionate but also butyrate

(Hippen et al., 2008; Linke et al., 2004). In a number of studies supplying PG or glycerol increased postpartum blood glucose levels and lowered plasma NEFA and BHBA. This effect was more readily observed in drenched cows than in cows receiving a diet that had incorporated PG or glycerol.

The immediate effects on milk production or composition appear to be limited especially when glycerol or PG is used to replace another non-starch carbohydrate (NSC) source. Application of these products under practical conditions depends thus greatly on the risk of ketosis; clinical or subclinical.

It is of interest to note that glycerol supplementation increased butyrate production without a concomitant increase in BHBA. Feeding of butyrogenic carbohydrate sources (e.g. molasses, beet pulp and lactose) or direct butyrate supplementation to dairy cows have been tested as a possible alternative strategy to improve energy status and reduce ketonuria (Defrain et al., 2004; Herrick, 2012 ;). However, neither the feeding of butyrogenic feeds nor direct butyrate infusion (at the rumen or abomasal level) have shown to increase blood glucose or insulin levels; rather the contrary (Krehbiel et al., 1992; Herrick, 2012). Direct glucogenic effects of butyrate are thus highly unlikely. Indirect positive effects may be possible through a glucose sparing effect of butyrate by shifting glucose metabolism from the liver to peripheral tissues (Kristensen et al., 2005). The possible role of butyrate in the etiology of periparturient health problems or the potential use as an additive remains inconclusive and deserves greater consideration; especially given the high blood levels of BHBA associated with dietary or rumen-generated butyrate and the importance of BHBA as an indicator of ketotic status.

Propionate supplementation in the form of Ca or other mineral salts has been suggested as a strategy to enhance supply of glucogenic precursors. However, results have been variable and often disappointing, possibly due to the relatively low levels of supplementation – especially relative to normal rumen propionate production (Overton and Waldron, 2004).

Although not a strict "additive solution", dietary changes that modify rumen fermentation and stimulating glucose supply and gluconeogenesis should be mentioned; notably since they will affect additive use. Most of these modifications are long term and look to stimulate production of propionate, quantitatively the most important glucogenic precursor. Propionate, lactate, and amino acids are considered to be important substrates for glucose synthesis however, recent work by Larsen and Kristensen (2013) question the importance of amino acids as glucogenic precursors in periparturient cows. This would leave lactate and propionate as the main glucose precursors. In order to increase their supply higher levels of concentrate should be fed raising the risk of rumen acidosis. Rations moderately rich in non-fiber carbohydrates (NFC) limit this risk and these types of diets have been suggested as an effective means to achieve improved metabolic profiles and health as well as post-partum DMI and milk production. However, more recent analyses would suggest that since most of the trials evaluating this approach confounded NFC with energy supply the data do not support this concept in favor of rations higher in structural carbohydrates (Overton and Waldron, 2004; Roche et al., 2013). On such diets the use of glucogenic additives (above) may be less frequent but are likely to be more effective.

Dutch workers have suggested that ration formulations that reduce the lipogenic-to-glucogenic nutrient ratio would improve the negative energy balance (NEB) and decrease plasma ketone concentration in early lactation. Such formulations would result in lower concentration of acetate and butyrate and higher propionate levels; with fat of dietary or body origin also being included as a lipogenic component (van Knegsel et al., 2007). These diet formulations - especially in short dry period situations - appear promising in improving energy balance and reducing plasma levels of NEFAs and BHBA while improving blood glucose and liver TAG levels. (Chen et al., 2014; van Knegsel et al., 2007; 2014).

Modifying Rumen Function

- **Direct-fed Microbials**

Utilization of additives that modify rumen function is of course not limited to the transition cow but their application seeks to meet specific objectives in support of the transition cow. The DFM most widely used in lactation diets of all ruminant spp. are fungal cultures notably various yeast varieties, primarily *Saccharomyces cerevisiae* and *Aspergillus* oryzae (AO) extracts (Amaferm). The exact mode of action of these additives remains unknown but they have been shown to work primarily at the rumen level by enhancing microbial fermentation and thus increasing substrate utilization. Among ruminal bacteria, two specific functional groups are stimulated, the fiber digesting and lactate utilizing bacteria. In addition, it has been demonstrated that AO extracts stimulate the growth of ruminal fungi that have been shown to play an important role in fiber digestion (Nagaraja, 2012). These additives increase rumen function by enhancing fiber digestion and reducing the transient post-prandial drop in pH. The combined effect of these DFM will assist cows to transition from high roughage diets to higher concentrate diets.

Feeding of transition cows' diets supplemented with DFM has shown an increase in milk production and dry matter intake (e.g. Nocek and Kautz, 2012; Baumgard et al., 2004). The increase in rumen fermentation and total VFA concentration or production should improve overall energy supply and metabolic profiles especially if propionic acid production is enhanced (Miller-Webster et al., 2002). An increase in relative proportion of propionic acid will stimulate gluconeogenesis. Improvements in levels in blood glucose, NEFA- and BHBA have been observed (Nocek and Kautz, 2012) confirming the potential positive effect on energy balance – especially postpartum. However, the response to DFM supplementation is variable in terms of production as well as blood parameters since some studies report no or limited effects. The absence of a response underlines the need to control the conditions under which these additives are applied, most importantly, diet composition, rumen pH and possibly overall stress (Chiquette et al., 2012; AlZahal et al., 2014).

DFM- or their cell wall components - have a well-recognized effect on the immune function in monogastrics animals. Mannan oligosaccharide (MOS) have been shown to act as a ligand offering competitive binding sites for gram-negative bacteria allowing removal from the digestive system. β-glucans have been shown to exhibit immune-modulatory effects. The effect of DFM on the immune status of cattle is much less studied. Recent results of feeding yeast fermentation extracts to transition cows would suggest that DFM can effectively induce the nonspecific immune system postpartum but do not seem to affect immunoglobulin concentrations (Zaworski et al., 2014). Nocek et al., (2011) demonstrated that enzymatically hydrolyzed yeast had a beneficial effect on SCC numbers in early lactation cows although the greatest effect was associated with a more advanced stage of lactation (8 – 14 weeks) rather than the period immediately post-partum.

A number of commercial products based on DFM or their products are on the market and they are indeed being used as immune-stimulants in dairy cow rations. Practical experience and proprietary reports are encouraging and would suggest that these are effective in stimulating - directly or indirectly - the immune system. Further, additional and impartial evaluation is needed.

- **Monensin**

Modification of rumen function needs to include consideration of monensin (although its utilization is limited to a few countries). Monensin – an ionophore - selectively inhibits gram-positive bacteria which results in a shift in rumen bacterial populations with a concomitant increase in propionate production. The increased production of propionate should stimulate gluconeogenesis and thus glucose supply. A meta-analysis of 59 studies on Monensin confirmed the increase in glucose (3%) and a concomitant decrease in NEFAs, ketone bodies (BHBA, acetoacetate) especially in the transition period thus demonstrating an improvement in the energy metabolism (Duffield et al., 2008). A direct effect of monensin on the immune

response has not been shown, consequently its effect in this area is probably mediated through its effect on circulating metabolites.

Improving Liver Function

The NEB that cows experience during the transition period is associated with the extensive mobilization of lipids from adipose tissue, causing marked elevations in circulating blood NEFAs and subsequently TG accumulation in the liver. In coping with the energy metabolism and the associated challenges this poses for the periparturient cow, the liver plays a key role. Liver function is impaired by TG accumulation and fatty liver is considered to be a determining factor in the reduction of normal liver function including gluconeogenesis and the metabolism of NEFAs as well as the elimination of endotoxins. Directly or indirectly it is also considered to play a major impact in the immune response. Reducing the extent and duration of fat accumulation in the liver is thus an essential part of dealing with the challenges associated with the transition cow.

The first step in dealing with the fatty liver syndrome remains the feeding program that maximizes postpartum energy supply, limits the NEB and reduces lipid mobilization. Subsequently, additives can be used to help the liver deal with the increased supply of NEFAs resulting from the obligatory fat mobilization through complete oxidation or the evacuation of esterified fatty acids as a constituent of VLDL. Export of VLDL from the liver is a slow process that can be stimulated though the feeding of choline. As a constituent of VLDL, choline supplementation will stimulate the production of the latter and therefore the transport of TG from the liver (Grummer, 2011).

The use of choline in a RPC has been tested and reviewed on a number of occasions (Grummer, 2011; Overton and Waldron, 2004). Overall, RPC has been shown to be effective in improving milk production (Sales et al. 2010) and reducing the impact of some of the parameters associated with the periparturient health challenges, most importantly liver TG levels (Piepenbrink and Overton, 2003; Zom et al., 2011). RPC has also been found to enhance specific gene expression that confirm the reduction in liver TG through improved FA processing and VLDL synthesis (Goselink et al., 2013). However, under practical conditions the effects remain variable. Some of this variability in response may be related to the quality of the rumen protection or supply of other dietary components notably the methyl donors contributing to the generation of choline (or its use). Unprotected choline in the stearate or chloride form has been shown to be degraded upwards of 98.0 % (Sharma and Erdman, 1989) and most publications assume a 100 % effectiveness of the fat coating which is highly unlikely.

Beside choline, ruminants have two main sources of dietary methyl donors: methionine and betaine. Studies concerning the effect of betaine are limited but methionine has been the subject of a consequential number of studies. In most modern dairy rations methionine is the first limiting amino acid for milk – but especially milk protein - production. Under those conditions methionine should play a minor role as a critical methyl donor. Nevertheless, considerable variations exist in rations concerning methionine supply and it is not clear as to what extent methionine has a sparing effect on choline's function as a methyl donor or vice versa. Methionine requirements are normally based on the established official tables (NRC 2001 or any other feeding system) based primarily on milk protein production. Its function as methyl donor is only indirectly considered, if considered at all.

Methionine deficiencies however, may interfere with the process required to produce choline from phosphatidylethanolamine or other reactions such as DNA methylation and histone modifications (thus affecting transcription of genetic information), an effect that may become more acute when choline is limiting. The work by Ardalan et al. (2009) demonstrated a degree of additivity when rumen protected methionine was supplemented to a control diet or a diet containing 15 g of protected choline. Reproductive performance, health status and milk production of dairy cows was improved by a combined supply relative to a single components or a negative control. Also the meta-analyses of Sales et al., (2010) concluded that on the basis of the estimated values for the metabolizable methionine, supplementary RPC

functions primarily as a methyl donor to spare methionine for milk protein synthesis thus explaining the primary positive effect of RPC on milk protein. The work by Osorio et al., (2014b) seems to confirm this as they demonstrated that methionine supplementation increased gene activation for the enzymes associated with methyl generation in periparturient cows. However, it is difficult to conclude on the exact relative role and effectiveness of the three methyl donors as the work by Davidson et al. (2008) demonstrated. These workers found no effect of rumen protected methionine or betaine on milk production or composition in early lactation cows fed a methionine deficient ration while RPC was effective in increasing milk production and milk fat or protein in multi-parous cows.

Improving Anti-Oxidant Status

It is now well accepted that the relatively large number and frequency of metabolic disorders associated with the transition period reflect in part a low antioxidant status resulting from the significant and fairly sudden increase in nutrient demands. Indeed, the final stages of pregnancy and onset of lactation result in an increased production and accumulation of reactive oxygen species (ROS) and consequently higher requirements for antioxidant (Spears and Weiss, 2008). The general relationship between oxidative stress and metabolic disorders during the periparturient period has been demonstrated by a lowered antioxidant status during metritis, retained placenta, acidosis, ketosis, milk fever and mastitis, (Celi, 2011). Also, it has been suggested that the impaired immune status, often associated with reduced liver function and increased inflammation associated with the periparturient cow results, in part, from an increase in the production of ROS. Consequently, it stands to reason that the supply of anti-oxidants and micro-nutrients to control the effects of oxidative damage should be considered as a routine component of the transition cow.

From a direct supplementation point of view, the (preventive) anti-oxidative system that can be affected through the diet seems limited to the supply of Vitamin E, β-carotene and selenium. In general, supplementation with these anti-oxidants improves immune function and health status in transition cows and an inadequate dietary vitamin E or Se decreases neutrophil function (Spears and Weiss, 2008). However, the productive and reproductive improvements following the supplementation with vitamin E and selenium vary with an important number of variables notably prior antioxidant status (enzymatic as well as non-enzymatic i.e. vitamins and Se), alternative dietary sources and - not in the least - the pro-oxidative stressors resulting from a sudden accelerated energy metabolism.

Improvements in liver function and how the cow deals with the inflammatory response can be improved through improvements in nutritional status i.e. the supply of nutrients that are not directly characterized as anti-oxidants but may stimulate parallel systems. This was explored by Osorio et al. (2014a) who measured a large set of variables that were directly or indirectly related to oxidative stress, liver function and inflammation in periparturient cows. These workers were able to demonstrate a change in oxidative stress associated with the supplementation with a methyl donor. Increasing methionine on a methyl-deficient diet improved *de novo* glutathione and carnitine synthesis in liver and, thus, increased antioxidant and β-oxidation capacity. This offers the possibility to reduce oxidative stress through other means than anti-oxidant supplementation.

CONCLUSION

The transition cow is confronted with a large array of physiological and nutritional challenges. Each of these can be addressed by a solution in the form of a specific additive. However, these additives must primarily be considered as support tools to a solid nutrition or feeding program that provides the basis for improvements in the cow's energy status through an enhanced dry matter and nutrient intake leading to better health, production, and reproduction post-partum. The selection of a specific additive needs to be based on a thorough analyses of the existing feeding program and the identification of the weakest link within the program.

Many of the additives that meet this objective need to be incorporated in the transition cow diet over the entire period in order to stimulate rumen fermentation and or energy metabolism. Since the liver plays a critical role in how the cow copes with the periparturient challenges improvements in liver function should be an important objective of the choice of additive. Direct or indirect effects on the immune system are equally important but direct effects of the available additives remain relatively poorly defined.

The additives available for incorporation in the transition cow diet affect a limited range of identified and quantified metabolic actions. Thus the use of a single additive is unlikely to cover all situations. The use of a combination of several additives in a supplement will provide ~~a~~ broader support and greater protection. Critical selection of these in one product or solution will ~~offers~~ the additional advantage of potential synergistic effects. Reports from practical experience seem to corroborate this multi-factorial approach.

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An Overview of Large Dairy Production Systems and Productivity Advances: 1970's to Present

Dan Loper
Loper Systems

In August of 1969, I left the United States Air Force as a Captain after working three years at the School of Aerospace Medicine in San Antonio, TX on the feeding systems for Gemini and Apollo space missions. I was employed by Western Consumer Feed Co. in California for the next three years. I was mentored by Fred Harshberger who was ahead of his time on feed buffers, organic trace minerals, copper and molybdenum relationships, and the Van Soest analysis and use of acid detergent fiber and lignin values in rations. He did more to reduce my learning curve regarding dairy nutrition than anyone else in my career. Almost all dairies in the western U.S. at that time bought mixed feed from a feed company. We used an IBM system 3 computer that cost $300,000 and took up half of a room for space. I traveled with a different feed salesman most days of the week from San Diego to Merced. In the early 1970's many dairies were moving to the San Janquin Valley or to Chino, Ca and flat barns where being replaced by herringbone barns. Since herringbone barns were so fast and feed intake was limited it became necessary to go to bulkier outside feed mixes that were fed with "turkey" feeding carts. Whole cottonseed and almond hull feeding become commonly used in the outside grain mixes. Feeding copra (coconut meal cake) for butterfat had to be abandoned because of its contamination with aflatoxin in the early 1970's.

In mid-July 1972, I left Western Consumers and started Loper Systems the first independent dairy nutrition consulting firm in the western states. I bought a Sanyo hand calculator for $240 since computers were a $100,000 to $300,000 per machine. I had $1000 between my checking account and savings account. I rented a small office in Chino, Ca. I needed advertising, but couldn't afford it, so I went to lunch with Bob McCune and Delores Mullins from "The Dairyman Magazine" and we made a deal that I would write an article on dairy nutrition every month in return for one third of a page of advertising for Loper Systems. I did that for four and a half years and it really paid off. We did a lot of testimonial ads with dairymen. I made 500 per month from my Amway business and that paid my 425 per month house payment. I sold Glen Sexton's Winnemucca mineral to feedlots and to some feed mills outside of California for buffering purposes.

In 1974 I saw my first feed mixing truck in the Imperial Valley at a 10,000 head feedlot that had flat commodity storage and a wheel loader, but no feed mixing mill. I was impressed by how fast and easy they fed all those cattle. I was excited as I headed home to Chino to start spreading the Gospel about dairymen mixing their own feed. They all had wheel loaders already. In the summer of 1974 Loper Systems put on a one day seminar on "How to Mix Your Own Feed" in Chino on a Tuesday and again in Tulare on a Thursday. Mixer trucks and national sales managers from Oswalt, BJM, Harsh and Crose were there in the parking lot to explain what their mixers could do and answered dairymen's questions. Bob Kennedy from Tulare, Ca was present to explain how a dairyman could contract to buy from a broker. He was one of the earliest feed brokers to sell directly to dairymen. Most existing feed brokers were afraid to risk direct sales to dairymen because it would jeopardize their feed mill business. We got some commodities from feedlots and big grain farmers like Salyer Bros. in Corcoran, Ca. In the beginning years commodities were difficult to find, but you cannot fight a tidal wave forever.

After 18 months in business, my family and I were almost starving so I sold my 1972 Pontiac and leased a 1974 Cadillac Coupe De Ville. Dairymen had been afraid to do business with me at first because they didn't think I could withstand the feed mill pressure and I would quit. The 1974 Cadillac with a DLOPER license plate changed their perception because in those days in California successful people drove Lincolns and Cadillacs. My men and I drove big cars in the 1970's and 1980's and they all had a LOPER license plate. This would not have worked in New Mexico and Texas.

In early 1975 I taught at Dr. Ensminger's Stockmen's School in San Antonio, TX. Several Arizona dairymen were in attendance. In July 1975 they telephoned me and asked me to come and meet with them in Phoenix and explain how I worked with dairymen who wanted to mix their own feed. Three dairymen volunteered to try my program- one quit after six months. I flew from Ontario, Ca to Phoenix in the morning and flew back home that night. We began taking the butterfat production trophies at the annual DHIA banquet. Within three years I was consulting with 23 dairies in Arizona with no computer yet. I hand calculated rations for protein, fat, fiber, calcium, and phosphorus. We sent the clients typed letters with the new rations and changes through the mail normally within one week. It was a lot of work. I would visit four dairies per day Monday-Friday and three on Saturday. I was exhausted by Saturday night.

In 1975 Loper Systems went to New Mexico and in 1977 we went to Idaho. In 1981 we went to Washington, Oregon, Colorado, and Texas. We eventually had five PH.D's and 150 plus dairies in the 1980's. In 1977 I bought my first dairy at Belen, NM. The next year I started another dairy at Muleshoe, TX. In 1981 I started a dairy at Dublin, TX and in 1988 I bought another dairy at Dublin, TX. By 1989 we were milking 3500 cows. After 30 years of dairy ownership I sold out in 2007. I was on the board of directors for Mid-AM for four and a half years.

In 1977 Loper Systems developed the 41 and 1 molasses product to replace 2 lbs. of cottonseed meal per cow per day. Its use became so popular that it spread from Ca. to AZ and all over the west and Midwest dairy states. It was a safe way to feed urea, and is still used today.
The use of lock up stanchions became widespread in the 1970's and 80's. Vet work and breeding moved outside the milking barn. It has remained a great management tool to the present day. Parallel and carousel milking barns have enabled dairymen to milk large numbers of cows on one dairy.

In 1981 I got a telephone call from Dr. Carl Alexander from Minnesota and he said that after 16 years he was on his own and had a new computer program that he wanted me to look at. It was named Dalex. We met and I told him it looked good for feed mills, but we would need some changes to use it in the consulting business. Over the next year we went back and forth and he developed the Dalex program that was used by many consultants. Apple computers came out at the same time, which made our work much easier.
Dr. Connor Jamison in Tulare, Ca developed the dairy comp 305 and Bliss Crandall in Provo, Utah also developed a computer record keeping system for dairymen. This signaled the end of the "card system" for cow records by Western Dairymen. Today you can use hand held electronics at cow side to look up a cow's history and performance and enter data.

Fax Machines came into use in the 1980's and improved communication in the dairy industry. Cell phones were expensive and cumbersome at first. All electronics got less expensive- computers, cell phones, copy machines and fax machines. Today a $400 computer can do more than a $300,000 computer could 40 years ago.

Herd monitoring systems are now available to alert dairy personnel if a cow is in heat or sick. Eating time and rumination can also be monitored.

Forage analysis labs developed in the 1980's and forage quality became the focus. Crude fiber was replaced by ADF and NDF. Feeding fats developed in the 1980's and is still evolving today.. By-Pass proteins received attention in the 1980's, such as distillers grain, soy based proteins, blood meal and fish meal. Amino acids lysine and methionine are used by some nutritionists. B-complex vitamins such as protected choline, niacin and biotin came on the market. Various forms of trace minerals and numerous yeast sources. Direct fed microbials improved gut health and reduced salmonella and e. coli loads in the lower gut and feces. Silage inoculants improved silage quality and shelf life. The "nickel rule" on cost of feed additives had a big effect on whether or not an additive made it in the market place.

Vertical feed mixers replaced many horizontal mixers because coarse roughage could be mixed without grinding first for dry cows, replacement heifers, and even milking cows. TMR's could get desirable feed intakes even with 160 RFV alfalfa hay.
BST use made more milk from fewer cows. Sometimes it created an over-supply and hurt the price paid for milk. Some consumers let it be known that they did not want BST milk. BST effects on reproductive performance were evident in heat stress environments.
Sexed semen has also been a mixed blessing since it added to our replacement heifer numbers and hence milk supply. It caused dairymen to raise cull rates since beef prices were high and replacement heifers were available at lower prices. Over supply of milk kills farm prices. Dairymen looking for Jerseys have benefited the most from sexed semen because it made Jersey replacement heifers available.

High feed prices, low milk prices, drought conditions and lack of irrigation water from 2009 through the present day caused drastic changes in rations for dry cows, replacement heifers, and even milking cow rations. Corn stalks, CRP grass, straw, cotton burrs, and haygrazer came into rations as never before. Corn gluten, distillers grains, and molasses with urea, were used as protein sources in those rations. Alfalfa hay levels were reduced in western dairy rations because of cost and availability.

Consolidation of dairy ownership continues to occur as many owners have two to five dairies. We have fewer decision makers to market our goods and services to. In my area of New Mexico, West Texas, and Central Texas we have 300 dairies and 75 nutrition consultants. Some consultants will take positions with dairymen who milk a huge number of cows and some will take industry jobs. Owners are more distant from their cows. Feed managers will become employed by dairy owners to purchase feed, handle feed shipping, monitor forage harvesting, sample forages, monitor feed mixing, evaluate feeders, and work with nutrition consultants. Several large dairies have already gone this route.

Stationary feed mixers will become more common. More effort will be made to reduce feed shrink especially in windy environments since feed cost is our largest cost.

Integration of crop farming and dairying will accelerate. Bankers are pushing this approach in recent years. Debt load per cow will decrease in order to survive hard times such as 2009 to 2013. Water availability will dictate the future of the dairy industry in the western states. In the Southwest, sorghum silage will continue to replace corn silage due to limited water for forage crops. Shredlage may become more common in the West. Will it work on sorghum silage and make the grain in silage more digestible? I have seen sorghum silage with as high as 28 % starch.

We lost antibiotic use in milking cow rations years ago. Consumer preferences are going to continue to dictate how we produce milk in the future. Prevention instead of reaction to sickness will become the NORM. The focus will be on building up the immune system of our dairy cattle. More plant extracts will be used for this purpose. Some of our current feed additives will be replaced by those plant extracts. This change is already happening currently in the poultry, swine, calf, and deer feeds. European countries have been researching and marketing these products for the least 20 to 30 years. We need to rely on their experience with these products as not all combinations will work together. We may get there in the future, but as for now, we are lacking knowledge on this subject. Most of these products have antioxidant and antimicrobial like actions and create a stronger immune system. Garlic keeps flies and mosquitoes off of horses and deer. Will it do the same with calves and thus reduce pinkeye? Will plant extracts replace Rumensin? Will effective mastitis tubes be developed from these plant extracts? This is an exciting new era.

In closing, integrity will always be an important part of the consulting business. You either have it or you don't. It is the key to the business and it will determine how long you last. I have seen many people come and go in this business over the past 45 years. When a consultant has to make a tough decision, the best decision for the client should always be the first and only choice.

Mycotoxin Management and its Importance in Feed Quality, Safety, and Maintenance of Reproductive Health and Milk/Beef Production in High Producing Ruminants

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SUMMARY

- Aflatoxin M_1 is the natural metabolite of aflatoxin B_1 and it has a high carry-over rate to animal products such as milk. Fresh milk is regularly checked for aflatoxin M_1; concentrations of M_1 above 0.5 µg/kg in the USA are considered undesirable and such milk cannot be used for products that go into the human food chain.
- The carry over rate of aflatoxins from contaminated feed into milk in dairy cows generally average 1–2%. However, in high yielding cows, which consume significant amounts of concentrated feeds, the carry over rate of aflatoxin M_1 into milk can reach up to 6.2%.
- Adsorptive performance of various feed additives can be very different even if they belong to the same mineralogical group. So called ‘‘pure’’ mineral binder feed additives are much more effective in *in vivo* in adsorption of aflatoxin B_1 than organic binder feed additives based on MOS and ß-glucans.
- The best practical way to control mycotoxin levels is to use rapid test kit systems for the analysis of mycotoxins in raw ingredients which are not yet in silos. A disadvatage of these rapid test kit systems is that they are usually not validated for analysis of various mycotoxins in silages and co-products.

INTRODUCTION

Mycotoxin contamination of dairy feeds is a worldwide problem for farmers as mycotoxins can increase the incidence of disease and reduce production efficiency in cattle (Coulombe 1993). Aflatoxins (B_1, B_2, G_1, and G2) are mycotoxins of major concern to the dairy industry. They are naturally occurring mycotoxins produced by the fungi species *Aspergillus flavus* and *Aspergillus parasiticus*. Most frequently, aflatoxins are found in maize and cottonseeds, and in their by-products. Less frequently, aflatoxins are encountered in soybeans and any type of distillers’ grains. The economic losses caused by aflatoxins are many and multi-component. First, all toxigenic fungi cause plant yield loss. Second, feed mycotoxin contamination reduces animal productivity due to health problems. Third, the contamination of crops and animal products (e.g., milk) is costly from a human health perspective. Fourth, additional losses associated with aflatoxins include the cost of prevention, sampling, mitigation, litigation, and research. The aflatoxin impact due to lost maize yield in the USA has been estimated around $225 million/year. Due to the constant global climate change and varying weather conditions in different regions of the world, the economic losses from discarded contaminated milk are unpredictable, yet real and significant. With most mycotoxins being carcinogenic to animals and humans, there is a wide legislation framework regarding their monitoring in the food supply chain. Aflatoxin B_1 is the most carcinogenic natural compound known (EFSA, 2004). Aflatoxin M_1 is the natural metabolite of aflatoxin B_1 and has a high carry-over rate to animal products such as milk. Fresh milk is regularly checked for aflatoxin M_1; concentrations of M_1 above 0.5 µg/kg in the USA are considered undesirable and such milk cannot be used for products that go into the human food chain. Contaminated milk must be discarded, and apart from the cost of lost milk revenue, the dairy producer must also suffer the cost of proper disposal of the contaminated milk. Aflatoxicosis is the disease caused by the consumption of high levels of aflatoxins by dairy cows. At low

levels of intake, usually there are no visual symptoms of aflatoxicosis, and as such the problem is often unnoticed. However, high concentrations of aflatoxins or prolonged exposure at low levels, cause visual symptoms in cattle, and especially in young calves. Beef and dairy cattle are more susceptible to aflatoxicosis than sheep and horses, whereas, young animals of all species are more sensitive to the effects of aflatoxins than mature animals. On the other hand, pregnant and growing animals suffer from aflatoxicosis less than young animals, but more than mature animals kept at maintenance (for example breeding males). Feed refusal, reduced growth rate, and decreased feed efficiency are the predominant signs of chronic aflatoxin poisoning. In addition, listlessness, weight loss, rough hair coat, and mild diarrhea may be observed in affected animals. Anemia along with bruises and subcutaneous hemorrhages are also frequent symptoms of aflatoxicosis. This disease may also impair reproductive efficiency, including abnormal estrous cycles (too short or too long) and increased abortions. Other symptoms include impaired immune system response, increased susceptibility to other diseases, and rectal prolapse. The diagnosis of aflatoxicosis is often difficult because of the variation in clinical signs, gross pathological conditions, and the presence of secondary infectious diseases due to a suppressed immune system. In addition, under commercial conditions, more than one mycotoxin may be present in any contaminated feed, and this makes definitive diagnosis of aflatoxicosis quite difficult. The effects of aflatoxin contamination as the disease progresses, depends upon the severity of caused liver damage. Thus, once overt symptoms are noticed, the prognosis is usually poor. Treatment should be directed at the severely affected animals in the herd, and measures should be taken to prevent further poisoning. Unfortunately, most lactating cows' positive for aflatoxins in milk will not exhibit strong visual symptoms, and as such, prevention is always the best way to tackle this problem. The carry over rate of aflatoxins from contaminated feed into milk in dairy cows is generally average 1–2%. However, in high yielding cows, which consume significant amounts of concentrated feeds, the carry over rate of aflatoxin M_1 into milk can reach up to 6.2% (Veldman et al., 1992). The aim of the experiment was to evaluate efficacy of different feed additives in the reduction of aflatoxin M_1 carry-over to milk.

MATERIALS AND METHODS

Sixty Holstein lactating cows (producing 13.61 to 54.43 kg milk) were fed aflatoxin B_1 contaminated corn grain (800 μg/kg) for a minimum of 3 days. If recalculated on TMR basis, the aflatoxin B_1 contamination was 170 μg/kg. Cows were divided into two replicates of 30 cows each. All cows were fed the same aflatoxin-contaminated total mixed ration (TMR) with either no additive (control) or one of eight additives at 0.5% of the TMR dry matter (DM). Milk samples were collected twice daily to evaluate changes in milk aflatoxin M_1 milk concentration. All changes were expressed as percentages and calculated relative to the control group which defined zero change.
Three measurements were:

- reduction in aflatoxin M_1 concentrations in milk
- reduction in aflatoxin M_1 excretion through milk
- reduction in aflatoxin transfer from feed to milk

Milk samples from the first replication were collected from each cow on the, 1st, 5th, 10th and 11th day of experiment. Milk collection days of the second replication were 15th, 20th and 21st. Samples were analyzed by HPLC according to the instructions provided in the "Afla M_1" Instruction Manual (VICAM®, L.P., Watertown, MA 01274). Data were analyzed by using SAS (Statistical Analysis Service, Cary, NC).
Calculations:

- Aflatoxin B_1 consumption (μg/day) = (DM consumed)*(aflatoxin B_1 level in DM).
- Excretion of aflatoxin M_1 (μg/day) = (amount of milk produced daily)*(aflatoxin M_1 concentration in milk). Milk aflatoxin M_1 concentration was measured as μg/l.
- Aflatoxin transfer from feed to milk (%) = 100*(excretion of aflatoxin M_1) / (aflatoxin consumption).

RESULTS AND DISCUSSION

Four of the eight additives resulted in significant reductions ($P < 0.05$) ranging from 34.98 to 40.39% for milk aflatoxin M_1 concentration, 36.36 to 52.28% for milk aflatoxin M_1 secretion, and 34.45 to 48.44% for aflatoxin M_1 transfer (Table 1). Dry matter intake (DMI) was significantly reduced ($P < 0.001$) by the consumption of aflatoxin B_1, while milk production was not affected during the same time period. Neither DMI nor milk production were affected by the addition of treatment products to the diet when compared to the control ($P > 0.05$). Adsorptive performance (adsorption capacity, selectivity, etc.) of the feed additives can be very different even if they belong to the same mineralogical group. Limitations of clay feed additives are that they accumulate in manure and, may be contaminated with toxic metals and dioxins which requires rigorous testing before use. Clay based feed additives may only bind mycotoxins other than aflatoxins only to a limited degree (Yiannikouris et al. 2004). Commercially available yeast cell wall based products, even of the same brand, were shown to differ in type and content of MOS and ß-glucan, as well as in ash content and mineral composition (Fruhauf et al., 2011). Our study is in agreement with Fruhauf et al., 2012 who claims that differences in the content and type of mineral clay components account for different binding capabilities of AFB_1. The adsorption rate at increasing amount of the toxin revealed big differences between ‘‘pure’’ mineral binder feed additives and yeast cell wall based products with mineral components added. Based on our results we can conclude that so called ‘‘pure’’ mineral binder feed additives were much more effective in *in vivo* adsorption of aflatoxin B_1 than organic binder feed additives based on MOS and ß-D-glucans.

Table 1. Percent reductions in milk aflatoxin concentration, milk aflatoxin excretion and milk aflatoxin transfer due to the addition of adsorbent products. A positive value indicates a reduction in aflatoxin transfer associated with use of the feed additive, while a negative value indicates an increase in aflatoxin transfer associated with use of the feed additive.

Additive (0.5% of DM)	Milk aflatoxin M_1 concentration, %	Milk aflatoxin M_1 excretion, %	Aflatoxin B_1 transfer from feed to milk, %
MTB-100®	-7.81	-6.71	-3.60
Ultra Sorb®	7.36	7.85	7.59
Mexsil®	6.62	8.00	7.19
Novasil+®	40.39*	42.59*	42.09*
TOXY-NIL® PLUS	34.98*	36.36*	34.45*
Condition ADE®	7.85	13.79	13.23
Astra Ben®	48.90*	52.28*	48.44*
Mil White®	46.49*	48.46*	44.55*

* Values are significantly different from zero ($P < 0.05$).

CONCLUSION

The best practical way to control mycotoxin levels is to use rapid test kit systems for the analysis of mycotoxins in raw ingredients which are not yet in silos. Different rapid test kit systems are validated for different mycotoxins and commodities which offer a very quick and effective way of raw material screening before they enter the feed mill. Once the levels are known, every feed mill can estimate the quality of its raw ingredients in terms of mycotoxin contamination and can effectively and more precisely (dosage adjustment) apply feed additives during feed production. Another strategy of mycotoxin risk management is to test for the presence of mycotoxins in finished feeds including TMR and silages. This method has some advantages and disadvantages. The most important advantage is that as every raw ingredient can bring its own mycotoxins into the finished feed and by only testing some raw ingredients by rapid test kits, some important raw ingredients whose inclusion is not high (5-10%) and which can still cause significant contamination of finished feed can be missed. Storage mycotoxin contamination (ochratoxins, aflatoxins) can be prevented by keeping temperature and moisture content in silos low whilst

grain is regularly aerated. In case perfect storage conditions cannot be guaranteed, use of mold inhibitor is highly recommended.

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Considerations of Gut Integrity in the Young Ruminant: A Review of Nutrition-Microbiota, Immune Function Interface using Novel Feed Additives to Enhance Sustainability and Productivity

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SUMMARY

- Many components of the gastrointestinal immune system develop postnatal.
- Dairy calves are highly susceptible to enteric disease and septicemia during the first few weeks of life as the gastrointestinal tract undergoes maturation.
- Bioactive compounds in colostrum and to a lesser degree milk influence maturation of the gastrointestinal immune system.
- Colonization of the gastrointestinal tract with microorganisms also further develops the gastrointestinal immune system.
- There is a progression in the microbial ecology of the gastrointestinal tract from facultative anaerobes to strict anaerobes, which will comprise over 99% of the microbes in the distal gastro-intestines throughout the life of the animal.
- Prebiotics and probiotics may hasten the microbial ecology progression.
- Butyrate may improve maturation of the gastrointestinal tract.
- Proteins from either hyper-immunized egg or plasma can improve disease resistance while the gastrointestinal tract matures.
- Calves can digest, absorb, and utilize the additional protein and energy early in life when fed greater quantities of milk replacer.
- The risk for some enteric diseases may be greater among calves fed greater quantities of milk replacer early in life.
- In contrast to early life, feeding greater quantities of milk replacer appears to improve post-weaning health.

INTRODUCTION

It is well documented that dairy calves are extremely susceptible to enteric diseases and mortality during the first few weeks of life. The latest reports from the USDA's National Animal Health and Monitoring System (NAHMS, 1993; 1996; 2007) report that the national mortality rate of heifer calves from 48 hours of life to weaning is approximately 7.8 to 10.8%. Producer perceived records indicate that scours account for 56.5 to 60.5% of all pre-weaned deaths. Approximately ¼ of all pre-weaned calves are therapeutically treated for scours, and the major causes of death from scours are either dehydration or the pathogen gains access to the blood and causes septicemia. The high incidences of disease indicate we have much to learn about improving gastro-intestinal disease resistance among pre-weaned calves. Very little is known regarding how the gastrointestinal immune system develops soon after birth; however, using enteric disease risk as an indirect measures indicates that is occurs rapidly, during the first few weeks of life. Nutrition, both directly and indirectly, can influence development of gastrointestinal immunity. Colostrum management, how much and the composition of milk or milk replacer fed, the use of various additives such as sodium-butyrate, prebiotics, probiotics, and proteins from hyper-immunized egg or plasma proteins can all influence the health of pre-weaned dairy calves. In addition, there are a few data that indicate that early life nutrition can have long-term impacts on leukocyte responses and disease resistance (Ballou, 2012; Ballou et al., JDS *In Press*; Sharon and Ballou, unpublished). There is a high incidence of

respiratory disease among dairy calves and is the main contributor to the high death losses, 1.8%, after weaning (NAHMS, 2007). This is an exciting area of research that needs to be addressed further.

WHY ARE CALVES SO SUSCEPTIBLE TO GASTRO-INTESINTAL DISEASE?

The calf is in a bit of a 'catch-22' situation early in life because it requires the passive absorption of many macromolecules from colostrum and milk, but this also increases the risk of translocation of pathogenic microorganisms. The gastrointestinal tract of many neonates undergoes a rapid maturation after parturition, and the timing of this depends largely on the species of interest. There are large gaps in our knowledge regarding how the gastrointestinal tract of a calf changes early in life; however, using gastrointestinal morbidity/mortality risk as an indirect measurement, the maturation occurs quite rapidly over the first few weeks of life. There are many components to the gastrointestinal immune system (Figure 1). Most of my discussion in this section was derived from animal models other than the calf, but the general principles can still be applied to the calf.

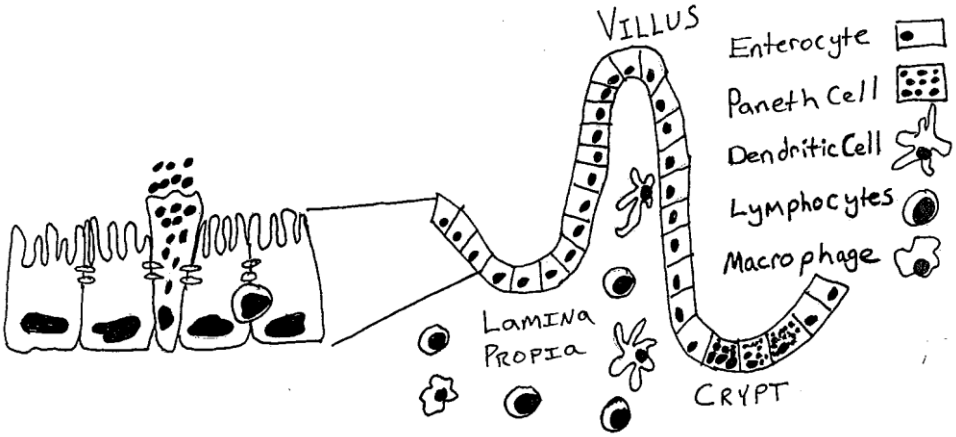

Figure 1. Schematic drawing of the small intestinal mucosa. The crypt-villus axis and common leukocytes found in the mucosa are shown on the right. The insert on the left is a magnification of the epithelial layer, depicting microvilli, tight junctions between epithelial cells, a goblet cell secreting mucus, and an intraepithelial lymphocyte.

The epithelial cells that make up the mucosal surface and the tight junctions between those cells form a **physical barrier** that prevents luminal contents from flowing directly into systemic circulation. A breakdown in the tight junctions increases the likelihood of infectious disease because of increased bacterial translocation. Goblet cells are one of the types of epithelial cells found in the gastrointestinal tract, and they produce mucus that creates a layer that covers most of the intestinal epithelium. This mucus layer forms an additional physical barrier against potential enteric pathogens. Additionally, the mucus layer contains many antimicrobial factors that were secreted from immune cells in the intestinal mucosa. These antimicrobial factors include: defensins, lysozyme, and sIgA, and their function is to limit the interactions of live microrganims with epithelial cells by creating a **chemical barrier**. Many leukocytes are found in the mucosa of the gastrointestinal tract as well as large lymphoid aggregates are localized in the submucosa of the distal region of the small intestines. These leukocytes contribute to the **immunological barrier** of the gastrointestinal tract. The majority of leukocytes found in the gastrointestinal (sub)mucosa contribute to adaptive immune responses and create memory that will help to prevent subsequent infections. Macrophages are found in the mucosa and could be involved in the clearance of some microorganisms, but neutrophils are rarely found in the mucosa and are only present in a pathologic state. Trillions of commensal microorganisms live in the gastrointestinal tract and they have a

symbiotic relationship with the calf. These commensal microorganisms are part of a **microbial barrier** that limits the colonization of the gastrointestinal epithelium with more potentially pathogenic microorganisms. These commensal microorganisms compete directly for substrates and space with the potentially pathogenic microorganisms and many of them produce antimicrobial factors and stimulate mucus production that further restrict potential pathogens from infecting the calf. These barriers work together to create a competent **Immune System** of the gastrointestinal tract. A defect in any of these components can increase the risk for infectious disease.

Many of the components of the gastrointestinal immune system begin to develop as early as the first trimester of gestation; however, further maturation of many of these barriers occurs only after birth (Guilloteau et al., 2009). This process of rapid intestinal maturation is known as "gut closure" and contributes to the **physical barrier**. The enterocytes, the nutrient absorptive cells that make up the majority of cells in the intestinal epithelium, are considered fetal-type at birth because they are largely vacuolated and can absorb intact macronutrients through pinocytosis. These fetal-type enterocytes are quickly replaced by more adult-like enterocytes. This process occurs from the proximal to distal intestines and from the crypt to the villus tip; therefore, even though the majority of the gastrointestinal tract may have undergone "gut closure" in the day and a half after birth there likely persist vacuolated, fetal-type enterocytes toward the villus tip of the lower regions of the intestines for a longer period of time. In addition to transcellular absorption of macromolecules, the gastrointestinal epithelium may also be more prone to paracellular absorption because of reduced tight junctions between the enterocytes. The mucus layer that covers the intestinal epithelium is dynamic and cannot be studied with traditional histological methods; therefore, very little is known regarding the postnatal changes in the mucus layer. Goblet cells respond to microbial exposure by increasing mucus secretion; therefore, it is conceivable that the mucus layer develops further during the post-natal period. Intestinal motility and the movement of digesta through the gastrointestinal tract can also reduce colonization of potentially pathogenic microorgansims, so a reduced intestinal motility can also contribute to the high incidence of enteric disease. Therefore, the **physical barrier** of the intestines is compromised during the early post-natal period and likely contributes to the high incidence of enteric disease and bacterial translocation.

The **chemical and immunological barriers** are also compromised during the early post-natal period. Paneth cells begin to develop during gestation; however, the number of Paneth cells and the antimicrobial secretions increase throughout life. Additionally, the adaptive arm of the immune system is naïve at birth and develops over the life of the animal as the calf is exposed and re-exposed to antigens. Therefore, sIgA concentrations and diversity are low and will remain low until the calf begins to develop its own active immunity. Antibodies from colostrum are known to recirculate back to the mucosa of the intestines, and can offer some immediate protection from enteric pathogens; however, the half-life of many passively derived antibodies is 1 to 2 weeks. Therefore, the gastrointestinal tract will become more susceptible to those specific microorganisms again until they develop their own active immunity against them. This is probably why many calves start developing localized enteric disease and scours during the 2nd or 3rd week of life. The fact is young animals will always be at an increased risk for infectious diseases until they develop their own active immunity. It's one of the benefits of getting older, the adaptive arm of the immune system becomes 'wiser' because of what it has been exposed to and experienced.

The calf *in utero* is developing in a relatively sterile environment and upon parturition and during the post-natal life they are exposed to a greater number and diversity of microorganisms. There is a progression in the microbial colonization of the gastrointestinal tract, with facultative anaerobes from the environment (ie: *Enterobacteriaceae*, *Streptococcus*, and *Staphylococcus)* dominating during the early post-natal period. There will be a switch to where strict anaerobes (ie: *Bifidobacterium*, *Bacteroides*, *Lactobacilli*, and *Clostridia*) will dominate and account for greater than 99% of the bacteria in the intestines for the rest of the animal's life. Therefore, the **microbial barrier** of the gastrointestinal tract is also compromised during early life and likely contributes to the greater incidence of enteric disease.

Therefore, from a systematic perspective, there are many holes in the gastrointestinal immune system defense during the early post-natal life. This greatly increases the relative risk for enteric disease. It is well known that what an animal is fed during the neonatal period will influence the development of the gastrointestinal immune system and enteric disease resistance. It should be noted that a lot more basic research on the development of the post-natal gastrointestinal immune system in calves is needed and should be a research priority.

MATURATION OF THE GASTROINTESTINAL IMMUNE SYSTEM AND PREVENTING PATHOGEN-HOST INTERACTIONS

A common management strategy in the dairy industry is to feed approximately 4L of colostrum within the first 6-12 hours of birth. Then calves are switched to either milk or milk replacer. It is well known that bioactive compounds in colostrum and transition milk directly influence the maturation of the gastrointestinal immune system. Our current colostrum management protocols are designed to ensure as many calves as possible get adequate passively derived immunoglobulins as possible. I don't want to down play the importance of passive transfer of immunoglobulins because it is essential in preventing systemic and local enteric diseases while the gastrointestinal tract matures; however, current colostrum management programs completely ignore the role that colostrum and transition milk play in the maturation of the intestinal immune system. Pasteurization of colostrum kills any potentially pathogenic microorganisms, but it also denatures a lot of other bioactive proteins found in colostrum, which could alter the ability of that colostrum to stimulate gastrointestinal maturation. This could explain why it is common to observe greater passive transfer of immunoglobulins among calves fed pasteurized colostrum. Enteric disease would likely be reduced if we fed calves to hasten the maturation of the gastrointestinal immune system. Adding feed additives to colostrum, milk, and milk replacer could improve maturation of the gastrointestinal tract. Most of our management decisions after feeding colostrum are aimed at reducing the interaction of potentially pathogenic microorganisms with the intestinal epithelial cells.

Butyrate is a short-chain fatty acid that is derived from microbial fermentation of carbohydrate in the rumen and the more distal portions of the gastrointestinal tract. It is well established that butyrate can influence proliferation of epithelial cells in many species. Increasing butyrate concentrations in the rumen of young calves improved rumen villi development. Additionally, feeding sodium butyrate increased proliferation and decreased apoptosis of epithelial cells in the lower portion of the gastrointestinal tract, which increased villi height and the potential absorptive area. Feeding sodium butyrate to pre-weaned calves also influenced digestive enzyme secretion and brush border expression (Gorka et al., 2009). Calves fed sodium butyrate had greater brush border expression of lactase, aminopeptidase A, and aminopeptidase N in the jejunum. Additionally, when sodium-butyrate was infused into the large intestines it stimulated the secretion of mucin, the major component of the mucus layer that helps protect the epithelial surface of the gastrointestinal tract (Barcelo et al., 2000). Lastly, butyrate may influence the integrity of the tight junctions between epithelial cells (Peng et al., 2007). Using a Caco-2 epithelial cell culture model, low concentrations of butyrate, 2 mM, increased transepithelial electrical resistance, a measure of integrity of the tight junctions. In contrast, higher concentrations of butyrate, 8 mM, decreased the transepithelial electrical resistance. Taken together, these data indicate that adding sodium butyrate to milk or milk replacer fed to calves may improve gastro-intestinal maturation and disease resistance.

Prebiotics, probiotics, and proteins from hyper-immunized egg or spray-dried plasma all have shown some merit in improving the resistance to enteric disease. Prebiotics are dietary components that are not easily digested by the calf, but are used by bacteria in the lower intestines to improve their growth. Probiotics are a vague term, but generally are live microorganisms that provide 'some' health benefit. At first glance this may seem bad, why would be want to improve the growth of bacteria in the lower intestines? As mentioned before, the intestinal tract is not sterile. Soon after birth, a wide range of bacterial species colonizes the gastro-intestinal tract of calves. Most of these bacterial species do not pose any immediate threat to the survival of the calf and in the past were called "good bacteria" and, of which, many of the common probiotic species are routinely classified as, including: *lactobaccilus* species,

bifidobacteria, *Enterocooccus faecium*, and *Bacillus* species. Remember that the microbial barrier of the intestinal tract soon after birth is colonized primarily by facultative anaerobes and subsequently becomes inhabited largely by strict anaerobes. Most of the probiotic microorganisms are strict anaerobes. Many of the probiotic species also have a direct bactericidal activity or compete with the more pathogenic microorganisms for limited resources. In addition, probiotics are themselves bacteria and they may "prime" the immune system of the calf by staying alert, as even the immune system recognizes the "good" bacteria as foreign. The common, commercially-available prebiotics available are the fructooligosaccharides (FOS), mannanoligosaccharides (MOS), lactulose, and inulin.

Data on the influence of prebiotics and probiotics alone on the health of dairy calves is equivocal. There are data that show improvements in reducing scouring and improving growth (Abe et al., 1995), whereas equally as many studies show no benefits to including either prebiotics or probiotics in milk (Morrill et al., 1995). The lack of a clear effect in calves is likely due to many environmental factors. Research does however support that many prebiotics and probiotics are generally safe and do not have any adverse effects on calf health of performance. In fact, most regulatory agencies around the world classify most prebiotics and probiotics as Generally Regarded As Safe (GRAS). Lastly, it is important to note that not all probiotic species and further, not all strains of a specific species, ie: not all *Lactobaccilus acidophilus* strains, behave similarly. Therefore, I would recommend only using probiotic species and strains that have been reported, through 3rd party research, to improve health and performance of calves. Additionally, viability/stability of the product should be confirmed as many of the probiotic species can become nonviable during processing and storage or undergo genetic drift.

Another strategy to reduce the interaction of pathogenic microorganisms is to feed egg protein from laying hens that were vaccinated against the very microorganisms that cause gastro-intestinal diseases in calves. The laying hens will produce immunoglobulins (IgY) and concentrate those proteins in their eggs, which can recognize the pathogen, bind to it, and prevent its interaction with a calf's gastro-intestinal tract. Inclusion of whole dried egg from these decreased the morbidity due to various bacteria and viruses. In addition to the use of hyper-immunized egg protein, spray-dried plasma proteins can improve gastro-intestinal health of calves. Spray-dried plasma is exactly like it sounds, plasma that is spray-dried to preserve the functional characteristics of the diverse group of proteins in plasma. The use of spray-dried plasma has been used for many years in the swine industry to improve the performance and health during the post-weaned period. The addition of spray-dried plasma proteins in milk replacer reduced enteric disease in calves (Quigley et al., 2002).

In 2010, my lab evaluated the effects of supplementing a blend of prebiotics, probiotics, and hyper-immunized egg proteins to Holstein calves from immediately after birth through the first 3 weeks of life (Ballou, 2011). Calves given the prophylactic treatment (n=45) were administered directly into the milk 5 x 10^9 colony forming units per day (from a combination of *Lactobacillus acidophilus*, *Bacillus subtilis*, *Bifidobacterium thermophilum*, *Enterococcus faecium*, and *Bifidobacterium longum*), 2 grams per day of a blend of MOS, FOS and charcoal, and 3.2 grams per day of dried egg protein from laying hens vaccinated against K99+ *Escherichia coli* antigen, *Salmonella typhimurium*, *Salmonella Dublin*, coronavirus, and rotavirus. Control calves (n=44) were not given any prebiotics, probiotics, or dried egg protein. All calves were fed 2 Liters of a 20% protein / 20% fat, non-medicated milk replacer twice daily. Prior to each feeding fecal scores were determined by 2 independent trained observers according to Larson et al. (1977). Briefly 1 = firm, well-formed; 2 = soft, pudding-like; 3 = runny, pancake batter; and 4 = liquid splatters, pulpy orange juice. The prophylactic calves refused less milk ($P<0.01$) during the first 4 days of life (57 vs 149 grams of milk powder). There were no differences in starter intake or average daily gain due to treatments. However, calves that received the prophylactic treatment had decreased incidence of scours ($P<0.01$) during the first 21 days of life (25.0 vs 51.1%). Scours were classified as a calf having consecutive fecal scores ≥ 3. The intensity of disease in this study was low and only 1 Control calf died during the experiment. These data support that a combination of prebiotics, probiotics, and hyper-immunized egg protein improve gastro-intestinal health and could be an alternative to metaphylactic antibiotic use. Future research should determine the efficacy of that prophylactic treatment in calves that

are at a higher risk of developing severe gastro-intestinal disease and subsequently death as well as investigate the mechanism(s) of action within the gastrointestinal immune system.

PLANE OF NUTRITION

The interest in the plane of nutrition that calves are fed during the pre-weaned period has increased primarily because data indicate that calves fed a greater plane of nutrition have decreased age at first calving and they may have improved future lactation performance (Soberon et al., 2012). More large prospective studies in various commercial settings should confirm that calves fed greater planes of nutrition during the pre-weaned period have improved future lactation performance. Most data on how plane of nutrition influences the health of calves during the first few weeks of life is limited to small, controlled experiments with fecal scores as the primary outcome variable (Nonnecke et al., 2003; Ballou, 2012). Many studies observed that the calves fed the greater plane of nutrition had more loose feces or greater fecal scores (Nonecke et al., 2003; Bartlett et al., 2006; Ballou et al., In Press JDS), while others reported no differences in fecal scores (Ballou, 2012; Obeidat et al., 2013). It is important to note, that no study has reported greater fecal scores among calves fed a lower plane of nutrition when compared to calves fed a greater plane of nutrition. It has been suggested that the greater fecal scores were not due to a higher incidence of infection or disease, but may be associated with the additional nutrients consumed. A couple of recent studies from my lab are confirming that calves fed greater quantities of milk solids early in life have greater fecal scores; however, when the dry matter percentage of the calves feces were determined there were no differences between calves fed differing quantities of milk solids (Liang and Ballou, unpublished).

It was unknown whether the digestibilities of nutrients of calves fed varying planes of nutrition were different during the first week of life. Decreased nutrient digestibilities would likely increase the risk of enteric disease because the increased supply of nutrients to the lower gastro-intestinal tract could provide a more favorable environment for pathogenic microorganisms to thrive. My lab recently tested the hypothesis that feeding a higher plane of nutrition during the first week of life would decrease the percentages of dietary nutrients that were digested and absorbed (Liang and Ballou, unpublished). Our justification for this hypothesis was that the reduced plane of nutrition during the first week of life would allow the gastro-intestinal tract time to adapt to enteric nutrition, without overwhelming the system. However, after conducting a digestibility trial with Jersey calves during the first week of life we had to reject that hypothesis. In fact, there was no difference in the percentage of intake energy that was captured as metabolizable energy, averaging 88% across treatments for the first week of life. We separated the first week of life up into 2 three-day periods and observed a tendency (P=0.058) for more of the intake energy to be captured as metabolizable energy during the 2nd period (85.9 versus 91.2 ± 2.0; 1st and 2nd period, respectively); however, the first period was likely underestimated because residual meconium feces would decrease the apparent digestibility. There was a treatment x period interaction (P=0.038) on the percentage of dietary nitrogen that was retained. The calves fed the greater plane of nutrition had improved nitrogen retention during the first period (88.0 versus 78.7 ± 1.20; P=0.004), but was not different from calves fed the reduced plane of nutrition during the second period (85.3 versus 85.0 ± 1.20; P=0.904). Most of the difference in nitrogen retention during the first period could be explained by differences in apparent nitrogen digestibility. It should be noted that apparent digestibility was likely more underestimated among the calves fed the restricted milk replacer during the first period because an equal quantity of meconium feces collected across the treatments during period 1 would underestimate the calves fed the restricted quantity of milk replacer more. The data from the digestibility study indicate that calves not only tolerate greater quantities of milk during the first week of life, but they incorporate those nutrients into lean tissue growth. The gastrointestinal immune system and implications to enteric health should further be investigated.

Over the past 7 years, my laboratory has conducted research to better understand the how plane of nutrition during the pre-weaned period influences leukocyte responses and resistance to infectious disease during the pre- and immediate post-weaned periods (Ballou, 2012; Obeidat et al., 2012; Ballou et al., In

Press, JDS; Liang and Ballou, unpublished; Sharon and Ballou, unpublished). The results indicate that plane of nutrition influences leukocyte responses of calves (Ballou, 2012; Obeidat et al., 2013; Ballou et al., In Press, JDS). In 2 studies, we reported that when calves were fed a lower plane of nutrition their neutrophils were more active during the pre-weaned period, as evident by increased surface concentrations of the adhesion molecule L-selectin (Figure 1) and a greater neutrophil oxidative burst (Obeidat et al., 2013; Ballou et al., In Press, JDS). After weaning the elevated neutrophil responses were no longer apparent in either of those studies. The exact mechanisms for the more active neutrophils among the low plane of nutrition calves are not known, but could be due to increased microbial exposure because of increased non-nutritive suckling, altered microbial ecology of the gastrointestinal tract, or reduced stress among the calves fed the low plane of nutrition. If the neutrophils are more active because of increased microbial exposure, calves fed a lower plane of nutrition could be at an increased risk for disease during the pre-weaned period if exposed to more virulent pathogens. Ongoing research in my laboratory is trying to understand the behavior and potential microbial exposure when calves are fed varying planes of nutrition and its influence on risk for enteric disease and immunological development. In fact, a few studies have shown that plane of nutrition during the pre-weaned period influence adaptive leukocyte responses. Pollock et al. (1994) reported that antigen-specific IgA and IgG_2 were reduced when calves were fed more milk. In agreement, Nonnecke et al. (2003) reported that less interferon-γ was secreted when peripheral blood mononuclear cells were stimulated with T-lymphocyte mitogens. However, not all data indicate that adaptive leukocyte responses are reduced when greater quantities of milk are fed; Foote et al. (2007) did not observe any difference in either the percentage of memory CD4+ or CD8+ T lymphocytes or antigen-induced interferon-γ secretion. All the leukocyte response data taken together suggest that calves fed lower planes of nutrition may have more active innate leukocyte responses driven by increased microbial exposure, which may explain the greater adaptive leukocyte responses. In a relatively sanitary environment this increased microbial exposure may improve adaptive immune development in the absence of clinical disease, but in a dirty environment it would likely increase the risk of enteric disease.

How plane of nutrition influences resistance to enteric disease is even less clear than how the leukocyte responses are affected. Quigley et al. (2006) reported that feeding a variable, greater plane of nutrition to high-risk Holstein bull calves, purchased from a sale barn and raised on bedding contaminated with coronavirus, increased the number of days calves had scours by 53% and also increased the number of days calves received antibiotics, 3.1 versus 1.9 days. In contrast, a more recent study reported that calves fed a greater plane of nutrition had improved hydration and fecal scores improved faster when they were challenged with *Cryptosporidium parvum* at 3 days of age (Ollivett et al., 2012). In a recent study from my lab, we orally challenged calves fed either a restricted plane or a greater plane of milk replacer at 10 days of age with an opportunistic pathogen, *Citrobacter freundii* (Liang and Ballou, unpublished). The calves fed the greater plane of nutrition had a greater clinical response to the challenge as evident by increased rectal temperatures ($P = 0.021$) and numerically greater peak plasma haptoglobin concentrations (511 versus 266 ± 108 μg/mL; $P = 0.118$). There also was a tendency for total mucosal height of the ileum to be increased among calves fed the greater plane of nutrition (921 versus 752 ± 59.1 μm; $P = 0.059$). The increased surface area of the lower gastrointestinal tract could partially explain the increased clinical response among the calves fed the greater planes of nutrition. Current data indicate that their likely is a pathogen:host interaction on the effects that plane of nutrition influence enteric disease resistance. Larger data sets with naturally occurring disease incidence and more experimentally controlled relevant disease challenges that are focused on the gastrointestinal immune system are needed before definitive conclusions on the role that plane of nutrition plays on enteric health of calves during the first few weeks of life. However, current data do not support that feeding greater planes of nutrition during the first few weeks of life are going to dramatically reduce enteric disease, so if you hear, “We have high incidences of disease and death in dairy calves because we restrict the quantity of milk they are fed” this is likely not true.

In contrast to health during the first few weeks of life, the plane of nutrition calves are fed during the pre-weaned period seems to be influence leukocyte responses and disease resistance among calves after they

are weaned (Ballou, 2012; Ballou et al., In Press, JDS; Sharon and Ballou, unpublished). Jersey bull calves that were fed a greater plane of fluid nutrition had improved neutrophil and whole blood *E. coli* killing capacities after they were weaned when compared to Jersey calves fed a more conventional, low plane of nutrition (Ballou, 2012). These effects were only observed among the Jersey calves in this study and not the Holstein calves. In a follow-up study, Jersey calves that were previously fed a greater plane of milk replacer had a more rapid up-regulation of many leukocyte responses, including neutrophil oxidative burst and the secretion of the pro-inflammatory cytokine tumor necrosis factor-α, after they were challenged with an oral bolus of 1.5×10^7 colony-forming units of a *Salmonella enterica* serotype *Typhimurium* (Ballou et al., In Press, JDS). The increased activation of innate leukocyte responses among the calves previously fed the greater plane of nutrition calves reduced (P=0.041) the increase in plasma haptoglobin and those calves also had greater concentrations of plasma zinc. The calves fed the greater plane of nutrition also had improved intake of calf starter beginning 3 days after the challenge (P = 0.039). These data indicate that the Jersey calves previously fed a greater plane of nutrition had improved disease resistance to an oral *Salmonella typhimurium* challenge approximately a month after weaning.

Recently, my lab recently completed a viral-bacterial respiratory challenge on calves a month after weaning that were previously fed either a restricted quantity or a greater plane of milk replacer (Sharon and Ballou, unpublished). Each calf was challenged intranasal with 1.5×10^8 plaque forming units of bovine herpes virus-1 per nostril and 3 days later were given either 10^6, 10^7, or 10^8 colony forming units of *Mannheimia haemolytica* intratracheal in 50 mL of sterile saline (n=5 per plane of nutrition and bacteria dose combination; N=30). Calves were observed for 10 days after the *Mannheimia haemolytica* challenge. The bovine herpes virus-1 challenge decreased calf starter intake by 21.2% in both plane of nutrition treatments. The *Mannheimia haemolytica* challenge further decreased calf starter intake, but again was not different between planes of nutrition (7.6%). All calves survived the entire observation period, but 2 calves were euthanized (were completely anorexic and did not respond to antimicrobial / anti-inflammatory treatments) 2 days after the end of the observation period and 2 calves died within a week of completing the observation period. All calves that died or were euthanized were previously fed the restricted plane of nutrition (1, 2, and 1 calves challenged with 10^6, 10^7, or 10^8 *Mannheimia haemolytica*, respectively). Necropsies of all 4 calves were consistent with severe pneumonia. Hematology and plasma data during both challenges indicated that calves previously fed the restricted quantity had a greater clinical response as evident by greater percentages of neutrophils in peripheral circulation (P=0.041) and plasma haptoglobin concentrations ($P \leq 0.097$). Therefore, the calves previously fed the restricted quantities of milk replacer had a more severe response to the combined viral-bacterial respiratory challenge, and the response was relatively independent of the *Mannheimia haemolytica* dose.

Therefore, the 3 studies from my lab are promising that early plane of milk replacer nutrition can influence the health of dairy calves within 1 month of weaning. Further, it appears that both enteric and respiratory health is improved with feeding greater planes of nutrition during the pre-weaned period. As was noted for enteric health during the pre-weaned period, larger data sets with naturally occurring disease and additional experimentally controlled challenges with leukocyte responses are needed before definitive conclusions can be draw. Further, it is of interest whether or not the improved health observed within 1 month of weaning would persist later into life and improve resistance to other diseases that are common during the life cycle of dairy cattle, including: gastro-intestinal, respiratory, metritis, and mastitis.

IMPLICATIONS

Dairy calves are extremely susceptible to disease in the first few weeks of life, which is likely related to the naïve gastrointestinal immune system of calves. Increasing the plane of nutrition in the first week or 2 appears to increase fecal scores, although the dry matter percentages of the feces were not different. Additionally, the digestibility of nutrients during the first week of life are great and does not appear to be impaired by feeding a greater quantity of milk replace solids. However, resistance to enteric disease during the first few weeks of life does appear to be influenced by plane of nutrition, but more data are

needed before more definitive conclusions can be made. Some early data are suggesting that feeding a greater plane of nutrition during the pre-weaned period may improve leukocyte responses and disease resistance of calves that extends beyond the pre-weaned period, but as with the effects of plane of nutrition on risk for enteric disease, more data are needed before we fully understand how early life plane of nutrition influences disease resistance later in life.

In addition to plane of nutrition, the use of feed additives such as: sodium-butyrate, prebiotics, probiotics, and proteins from hyper-immunized egg or spray-dried plasma were all shown to influence gastrointestinal maturation and/or reduce the incidence of gastro-intestinal disease.

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NDF Intake = 1.25% * Body Weight … What Are We Missing?

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SUMMARY

- Dry matter intake (DMI) for dairy cattle can be related to feed NDF level.
- Since the 1980's, ruminal NDF digestibility (dNDF) has been used in ration formulation. It has been reported that a unit increase in dNDF corresponds to a 0.25 kg increase in 4% fat corrected milk.
- When dNDF is increased, undigested NDF is correspondingly decreased. More recent observations suggest that the proportion of forage dry matter represented by undigested NDF (NDF_{u30}) is a major determinant of DMI.
- Understanding factors which cause a divergence between estimated NDF_{u30} and actual NDF_{u30} is critical. These include: improper ash correction in the NDF analysis, inherent variation in digestibility testing, increased rumen passage rate, and ruminal acidosis.

INTRODUCTION

In a recent conference proceedings, Mertens (2010) appropriately stated: "Although the basic biological principles by which fiber affects intake have not changed, our knowledge about the subtle ways in which the characteristics of fiber impact intake regulation and our ability to speculate about the dynamic mechanisms that affect the relationship have changed during the last 15 years.". A traditional view is that NDF intake can be expressed as a fraction of body weight. Waldo (1986) suggested that cell wall concentration (i.e., NDF) of forage diets is the best single chemical predictor of DMI by ruminants.

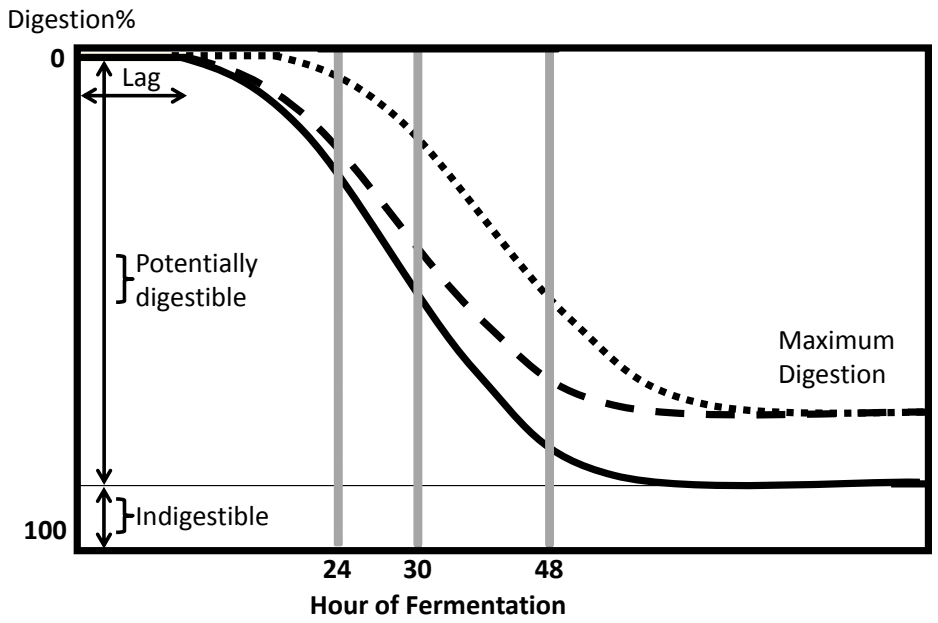
It is clear that increasing NDF digestibility increases intake (Oba and Allen, 1999). This understanding led to forages being characterized by NDF digestibility (NDF_d; % of NDF). Allen (2000) concluded that "Digestibility of NDF measured *in vitro* or *in situ* using a constant incubation time was a significant indicator of the filling effects of NDF …".

A common convention is to use a 30-hour *in vitro* incubation to estimate NDF digestibility (NDF_{d30} % of NDF). Feeds with higher NDF_{d30} (% of NDF) are generally found to promote more intake. However, the effect of the potentially digestible NDF fraction on gut fill was not clear when evaluated by Allen and Mertens (1988).

Jones and Siciliano-Jones (2013) proposed that the proper characterization of fiber related to intake is the pool size of undigested fiber (NDF_u; % of DM). Following the convention presented above, the pool size of undigested fiber after a 30-hour *in vitro* incubation was introduced (NDF_{u30}©; % DM, Copyright FARME Institute, Inc).

Hall (2013) discussed a debate over which incubation time is most appropriate for estimating NDF digestibility (see Figure 1). The 2001 NRC uses a 48 hour incubation time to estimate energy derived from NDF. Our purpose is to estimate "gut fill" which must take into account passage rate. The 30-hour incubation time point seems appropriate given static *in vitro* fermentation and a standard particle passage rate. If the remaining particles have not been fermented or passed, they contribute to gut fill.

Figure 1. Rate of digestion as seen at different time points given different digestion rates and lag times (Hall 2013).



It is important to differentiate between the terms of "undigested" and "indigestible". The former refers to the ability to be digested given a finite time. In this case, 30 hours. The latter refers to the ability to be digested given infinite time. Usually this is estimated at 240 hours of incubation in rumen fluid.

Examining previous work on NDF digestibility, expressed as percent of NDF fraction, we can substitute the measure of NDF_{u30} expressed as pool size. Previous work that increased NDF digestibility in diets also decreased NDF_{u30}, usually without noting it. For example, Allen (2000) notes that "DMI by cows will be less limited be distention in the gastrointestinal tract as NDF digestibility increases." The concept of fiber digestibility impacting gut fill is not new. However, the proper representation and utilization of NDF_{u30} is new.

NDF_{u30} IN RATION DESIGN

NDF_{u30}© is proposed as an indicator of "gut fill" to be used in designing certain dairy cow rations (Jones and Siciliano-Jones, 2014). First, it is only appropriate to discuss NDF_{u30} in rations where DMI is limited by gut fill. This is typical of intakes during peak production (Mertens, 2010) Situations where DMI is limited by low energy requirement or acid load will likely not respond to manipulating NDF_{u30} content. NDF_{u30} acts as a gut fill factor only when fed particle size is large enough to inhibit passage from the rumen. The threshold particle size allowing passage from the rumen appears to be 2-4 mm (cited by Allen and Mertens, 1988). Consequently, undigested NDF in particles below this threshold will not be expected to contribute to gut fill as they are not retained in the rumen. Therefore, we propose calculating the pool size of NDF_{u30} only on feeds that have a particle size above 4 mm. In general, only forages and certain large by-products (e.g., whole cotton seed) are included in the gut fill calculation.

Our basic procedure is to calculate the NDF_{u30} content in the forage portion of a ration for a high producing group of dairy cows. As a starting point, high producing large Holstein cows appear to eat about 6.2-6.5 pounds of NDF_{u30} per day. However, what is important is how this NDF_{u30} content changes over time relative to DMI (Jones, 2014). If a forage or ration change results in increased NDF_{u30} in the

proposed ration, there is a high probability that DMI will decrease such that the group's actual threshold of NDF_{u30} capacity is not exceeded.

Using the above procedure requires two assumptions. First, it is assumed that gut fill (i.e., NDF_{u30} content) is the most constraining factor in the ration. Second, a forage base (including all significant sources of NDF_{u30}) must be the initial component of ration design. Designing a ration with NDF_{u30} starts with a forage base that does not violate gut fill. This is also intuitive since a ration should be first balanced for the rumen and then for the animal.

It is tempting to discuss NDF_{u30} as a percent of ration dry matter. This has benefits for ration formulation but does not reflect the underlying subject that gut fill is a pool size issue. Let's start with a farm specific assumption that the highest cows have not historically consumed more than 6.3 pounds of NDF_{u30}. Problems arise when a group is balanced for a DMI which is below that consumed by the highest producing cows. For example, a group ration might be balanced for 53 pounds of DMI. However, the highest producing cows might be eating 70 pounds of DM to support peak milk production. A typical calculation is to determine the percentage of NDF_{u30} to ensure that the highest producing cows are not challenged with more than 6.3 pounds of NDF_{u30} intake. In this case, the base ration needs to be 9% NDF_{u30} (6.3 # NDF_{u30}/70 # intake). Conversely if NDF_{u30} percentage is calculated from the group intake (6.3 # NDF_{u30}/53 # intake), the NDF_{u30} content will increase such that the highest producing cows will reach their fill capacity at a reduced DMI. Recommendations for NDF_{u30} as a percentage of DM should be avoided for this reason. This calculation is only useful in determining if the base ration for a group will support the highest producing cows.

Common ration design rules can violate the gut fill capacity of cows resulting in lower milk production. For example, a common ration feature is inclusion of 3 pounds of WCS (DM basis) in all diets. Assuming that WCS is 40% NDF_{u30}, WCS contributes 1.2 lbs of NDF_{u30} to these diets. In a year when NDF digestibility of CS corn silage is poor (e.g., NDF_{u30} increases from 15% to 18%), a ration containing 20 pounds of corn silage will see an increase of 0.6 pounds of NDF_{u30}. Without adjusting the WCS or the corn silage inclusion rates, the high producing cows will have DMI limited by gut fill due to excess NDF_{u30}.

A common consequence of exceeding the gut fill capacity of high producing cows is lower than expected peak production. When DMI is limited by gut fill, the highest producing cows will be impacted the most due to the inability to consume sufficient DMI. When older animals are peaking poorly compared to their younger cohorts, especially when persistency is high, a gut fill problem should be suspected.

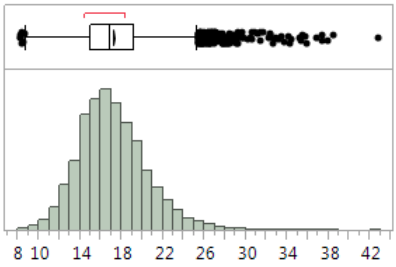
DISTRIBUTION OF NDF_{u30} IN FORAGES

Figure 2 contains the distribution of NDF_{u30} for both corn silage and hay crop silage in the Cumberland Valley Analytical Services database. Corn silage has a mean NDF_{u30} value of 17.2%. For hay crop silage, the mean is 23.9% NDF_{u30}.

Figure 2. Distribution of NDF_{u30} content for corn silage and haylage observed in the Cumberland Valley Analytical Service database. Provided by R. Ward, 2013, Cumberland Valley Analytic Services.

Distributions

uNDF30 (cs)

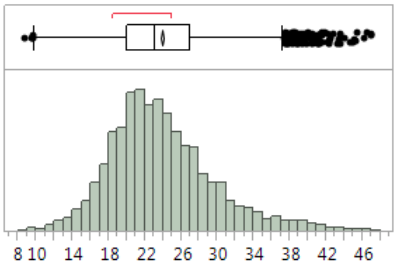


Quantiles		
100.0%	maximum	42.7673
99.5%		28.3225
97.5%		24.5807
90.0%		21.4509
75.0%	quartile	19.1271
50.0%	median	16.89
25.0%	quartile	14.969
10.0%		13.391
2.5%		11.6215
0.5%		9.98573
0.0%	minimum	8.17646

Summary Statistics	
Mean	17.225373
Std Dev	3.3169967
Std Err Mean	0.0310189
Upper 95% Mea	17.286176
Lower 95% Mean	17.164571
N	11435

Distributions

uNDF30 (hc)



Quantiles		
100.0%	maximum	47.0018
99.5%		42.4452
97.5%		38.1842
90.0%		31.2629
75.0%	quartile	26.9821
50.0%	median	23.1107
25.0%	quartile	20.0685
10.0%		17.5634
2.5%		14.5019
0.5%		11.6742
0.0%	minimum	8.65938

Summary Statistics	
Mean	23.901011
Std Dev	5.7100741
Std Err Mean	0.0971444
Upper 95% Mea	24.091477
Lower 95% Mean	23.710545
N	3455

The variance seen in these distributions suggest fairly large gut fill differences. First, it becomes clear why high corn silage diets generally result in less gut fill. The average corn silage sample has nearly 7 percentage points less NDF_{u30}. A ration that contains equal amounts of average corn silage and average haylage with a constraint of 6 pounds of NDF_{u30} will contain 29 pounds of forage. Conversely, a diet with 80% average corn silage and 20% average haylage will allow 32 pounds of forage.

A common scenario occurs when a growing year results in lower fiber digestibility (i.e., higher NDF_{u30}). Consider again a 80:20 corn silage:haylage diet when the NDF_{u30} changes from an excellent corn silage (25% quartile; 14.97 % NDF_{u30}) to a poor corn silage (75% quartile, 19.12% NDF_{u30}). The NDF_{u30} content of the diet will increase from 6 to 7.3 pounds. If our group was eating 66 pounds of DM (9% NDF_{u30}), the intakes will probably decrease to 54 pounds due to increased gut fill.

A related topic is the accuracy of NDF digestibility as measured in the laboratory. One should remember that digestibility testing has been common since the 80's (Nocek and Russell, 1988) and was intended to be a qualitative test for ranking forages since the variability is much higher than typical chemical analyses performed on forages. Hall and Mertens (2012) reported that within a given laboratory, 95% of the digestibility results for a given forage sample fall between ± 4.9% NDFD from the mean. If we use a typical forage consisting of 40% NDF and a 50% NDFd, then the NDF_{u30} will be 20%. If the NDFd measure varies from 45 to 55% then the NDF_{u30} will vary from 18 to 22%. This does not take into account the variation inherent in NDF chemical analysis which would further increase the range of values. Using NDF_{u30} as a gut fill index is consistent with the notion of a qualitative index.

WHEN DOES PREDICTED NDFu30 ≠ ACTUAL NDFu30?

As forage analysis evolves, it is becoming more biological than chemical in nature. For example, measuring starch content is a simple chemical analysis. Conversely, estimating starch availability requires mimicking the biology of starch digestion. This is also true for NDF digestibility. To correctly apply

NDF_{u30} in ration design, it is important to explore scenarios where the predicted NDF_{u30} does not properly estimate the biological NDF_{u30}.

As an example, consider the haylage sample shown below. The NDF_{u30} is 27% of the DM. If our new diet design calls for 2 pounds of NDF_{u30} from haylage, we will limit inclusion in the diet of this haylage to 7.5 pounds of DM. In this scenario, the cows will almost certainly increase DMI. Why? The NDF_{u30} is not really 27%. This analysis demonstrates a classic example of NDF which is not corrected for ash contamination. Looking closer at this sample, there is a 9 point difference between aNDF and aNDFom. Further, the NDF_{d30} and NDF_{u30} are calculated from aNDF (13.7% + 27% = 40.7%). From a typical haylage, the NDF_{u30} is overestimated by approximately 6-7 points. A better estimate is 21% NDF_{u30} which now allows an inclusion of 9.5 pounds of haylage in our example diet. When there is high ash content (> 3%) in the NDF fraction, the undigested portion will be overestimated when calculated using aNDF which is not corrected for ash content.

Figure 3. Example fiber analysis in a forage sample that contains ash contamination in the NDF fraction.

FIBER	% NDF	% DM
ADF	89.3	33.1
aNDF		**40.7**
aNDFom		31.8
NDR (NDF w/o sulfite)		
peNDF		
Crude Fiber		
Lignin	18.32	7.46
NDF Digestibility (12 hr)		
NDF Digestibility (24 hr)		
NDF Digestibility (30 hr)	**33.8**	**13.7**
NDF Digestibility (48 hr)		
NDF Digestibility (240 hr)	46.5	18.9
uNDF (30 hr)	66.2	27.0
uNDF (240 hr)	53.5	21.8

A second scenario which will overpredict the gut fill impact of forages is finely chopped diets. NDF_{u30} calculated *in vitro* is independent of passage rate. When passage rate increases, the amount of particles remaining in the rumen at a specific time decreases. Consequently, excessive NDF_{u30} intake can be an indicator of increased NDF passage. Another documented scenario is that passage rate changes with the animal's cold stress. Hence, gut fill capacity may change during periods of cold stress.

Ration characteristics that reduce fiber digestibility constitute a third scenario where gut fill is higher than predicted. Most common is increased acid load that inhibits fiber digesting bacteria. Low ruminal pH from highly fermentable feeds can decrease rate of fiber digestion and increase the filling effect of the diet (Allen and Mertens, 1988). Recently, ration starch has been a focus as a dietary component that lowers ruminal pH. This focus has ignored the reality that digestible NDF can also be highly fermentable and contribute to acidosis. In a recent popular press summary, Fredin (2014) showed that replacing starch with non-forage fiber sources did not change rumen pH. It should not be surprising that low starch diets combined with other sources of highly fermentable carbohydrate can result in low rumen pH which will depress fiber digestion. This, in turn, increases actual NDF_{u30} and the gut fill characteristics of the diet.

Differences in particle retention time for different types of forage NDF can cause predicted NDF_{u30} to not correspond to actual NDF_{u30}. In general, NDF in legumes is thought to have less filling effect than NDF in grasses (Oba and Allen, 1999). An example of this effect was seen in a study to examine perennial ryegrass silage compared to alfalfa silage where the alfalfa silage was found to support greater DMI (Hoffman et al., 1998). Recalculating their data into a gut fill context, the alfalfa silage was 20.9% NDF_u while the perennial ryegrass was 16.8% NDF_u as a percent of DM. However, in this case, the cows consuming the alfalfa silage ate nearly 5 pounds more DM than the perennial ryegrass. The differing gut

fill effect of different forages types argues for monitoring the gut fill effects in diet specific scenarios (Jones, 2014).

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Interactive Effects of Trace Mineral Supplementation on β-Adrenergic Agonist Response in Feedlot Cattle

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SUMMARY

- Recent research indicated that trace mineral supplementation may alter the biological response of a β-adrenergic agonist such as ractopamine HCl and zilpaterol HCl in cattle.
- Evidence indicated that zinc can interact with β-adrenergic receptor affecting receptor function and downstream activation.
- Independent of the β-adrenergic receptor function, zinc appears to impact protein synthesis and degradation through the mTOR pathway.
- Chromium has profound effects on glucose metabolism at the skeletal muscle level.
- Increased glucose availability caused by chromium has positive effects on skeletal muscle hypertrophy during a β-adrenergic receptor-mediated growth response in cattle.

INTRODUCTION

Beta-adrenergic agonists (β-AA) are commonly used in the beef cattle feedlot industry to improve growth performance and carcass characteristics through increased protein synthesis and decreased protein degradation (Mersmann, 1998). Beta-adrenergic agonists have also been reported to increase lipolysis and decrease lipogenesis in adipose tissue (Mersmann, 1998). These β-AA, work through an interaction with the beta-adrenergic receptors (β-AR). Zilpaterol HCl (ZH) was a commonly used β-AA in cattle that primarily bound with the β2-AR, which is the most predominant β-AR found in cattle muscle and adipose tissue. Via a secondary messenger signal cascade event, cyclic adenosine monophosphate (cAMP) is activated thereby resulting in protein accretion and lipid catabolism.

Overstimulation of the β-ARs by β-AA has been reported to result in receptor desensitization (Lohse et al., 1990; Waldo et al., 1983). Receptor desensitization results in a down regulation of adenylate cyclase catalytic activity resulting in a reduction of cAMP synthesis (Pippig et al., 1993). When the β-ARs become desensitized, they are sequestered within an intracellular vesicle, thus losing the ability to induce signal transduction (Lohse et al., 1990; Waldo et al., 1983).

Research has shown that the β2-AR potentially has multiple allosteric binding sites for zinc (Swaminath et al., 2002). According to Swaminath et al, (2003), there are two main binding sites for Zn on the β-AR; one affects the agonist's ability to bind to the receptor, while the other affects the antagonist's ability to bind to the receptor thus increasing cAMP production. Zinc also regulates adenylate cyclase (AC) and cyclic nucleotide phosphodiesterases (PDE) which are involved in the synthesis and degradation of cAMP after the β-AR is activated (Haase and Rink, 2011) . Several studies have reported the catalytic activity of AC is inhibited by Zn; however, the mechanism responsible for this phenomenon is still unknown (Brown et al., 2002; Klein et al., 2002; Klein et al., 2004). Von Bulow et al. (2005) reported the addition of Zn to cellular lysate inhibits cyclic nucleotide degradation, indicating increases in cellular Zn will block PDE activity.

Little is known about how the combination of ZH and Zn might influence the β-AR's ability to produce cAMP, and its regulation of mRNA and protein synthesis.

Cell signaling within the body is a complex system that depends on many different molecules, minerals, and hormones. A micromineral that has many different roles throughout the body, including cell signaling, is zinc. Zinc plays an essential role in bodily functions; however, much is still unknown about all this micromineral's actions. The availability of zinc varies between tissues, with the majority of the molecule being found intracellularly (Haase and Maret, 2003). Tissues vary in concentration, from 10 µg/g in brain tissue to as high as 100 µg/g in bone (Mills, 1989); however, plasma concentrations are much lower at approximately 1 µg/g (Haase and Maret, 2009). The majority of all zinc is not free ions, but is bound to different proteins (Haase and Rink, 2011). This means that "free zinc" is extremely hard to find and is typically just referred to "free" because it has the possibility to be utilized, even if bound to a certain protein.

As mentioned earlier, zinc is a highly controlled ion found throughout the body. The reason behind this is its capacity to bind many different proteins. Some of these proteins are regarded as zinc binding proteins, such as metallothionein. Metallothioneins are similar to chaperone proteins that bind to divalent metal ions, like zinc. According to Peroza et al. (2009), the structure of the metallothionein is a ubiquitous protein containing cysteine-rich regions. The zinc-binding site of a certain metallothionein comprises two cysteine and histidine residues with a zinc rich region held in the middle with up to nine cysteine residues (Peroza et al., 2009). A common characteristic among metallothioneins is their low molecular weights (Takahashi, 2012). The low molecular weight may be of importance when crossing membrane spaces (Takahashi, 2012).

It is speculated that some of these metallothionein proteins have a specific function to perform with the necessity of binding zinc, while some serve as reservoirs for zinc to bind (Maret 2009). The latter suggests that metallothionein proteins may exclusively have a role in chaperoning zinc. This idea would support the belief that zinc is a tightly regulated ion and chaperone proteins, such as metallothionein, would play a key role in this. According to Ye et al. (2001) a zinc binding metallothionein is involved in trafficking zinc across the membranes of the mitochondria. In this case, metallothionein would serve as a transport protein, and not just a reservoir for zinc. It is quite possible that zinc aids in cellular respiration and is needed in the mitochondria as Ye et al. (2001) reports that if zinc is sequestered from the intermembrane space of the mitochondria, the cell loses respiratory function; however, once metallothionein is reintroduced to the cell and allowed to maintain the correct zinc concentrations, cellular respiration is regained.

Nuclear Action of Zinc

Zinc's role in DNA replication is well documented. According to MacDonald (2000), zinc is present throughout the nucleus, as well as the nucleolus and chromosomes. The action of zinc in these areas is to stabilize the structure of DNA, RNA and chromosomes (WU and Wu, 1987). According to Wu et al. (1992) zinc forms metalloenzymes that are associated with DNA and RNA synthesis, such as RNA polymerase. Other metalloenzymes that Wu and Wu (1987) found that contain zinc are reverse transcriptase and transcription factor IIIA. Zinc's presence in these metalloenzymes is crucial to the functionality and stability of the enzyme and helps make structures necessary for biological function of the enzyme (Chesters 1991).

A commonly known structure that zinc belongs to in these enzymes are zinc finger domains. The structure itself comprises a zinc ion, which causes a loop to form in the polypeptide chain by linking cysteine and histidine amino acid residues (MacDonald 2000). Vallee and Auld (1995) found that these zinc finger domains are essential for proper motif function by providing the enzyme a direct binding site to DNA sequences in eukaryotic cells. Zinc's profound involvement in regulatory proteins causes a necessity for zinc in the cell. Bunce (1994) found that difficulties that may arise from zinc deficiency include defects in

embryogenesis, growth and differentiation, and regulation of the superfamily of nuclear hormone receptors.

β-agonist Receptor Desensitization and the Allosteric Binding of Zinc

Stimulation of the β-receptor by an agonist has the possibility of causing desensitization of the receptor. There are two accepted reasons for desensitization of the βAR: one being phosphorylation of the intracellular portion of the receptor by kinases, two examples being PKA and β-adrenergic receptor kinase (βARK) (Acute short term effect) and the other being internalization of the βAR (Chronic long term effect). The short term, more rapidly desensitization of the β-adrenergic receptor is caused from uncoupling the Gs protein from the receptor (Hausdorff et al., 1990; Pippig et al., 1993). Hausdorff et al. (1990) and Benovic et al. (1987) reported that the receptor is phosphorylated by PKA and βARK, this phosphorylation dissociates the receptor from the GS protein, and internal signaling is down regulated. Another protein also is thought to be involved in this form of desensitization known as β-arrestin. Pippig et al. (1993) showed that in Chinese hamster ovary cell lines, desensitization first begins with phosphorylation of the βAR by βARK which then precedes to binding of the internal portion of the receptor to β-arrestin. The group showed that as the concentration of β-agonist (isoproterenol) increased, the amount of desensitization also increased, leading to the conclusion that overstimulation of the receptor is what appears to be causing the loss in functionality of the receptor do to βARK and β-arrestin's involvement. Pippig et al. (1993) and Lohse et al. (1990) theorized that down regulation of receptor signaling at low concentrations was due to PKA phosphorylation of the receptor alone. Both of the above types of desensitization result in down regulation of adenylyl cyclase, which disallows the receptor from sending an internal cell signal via cAMP (Pippig et al. 1993; Lohse et al. 1990; Hausdorff et al. 1990).

The second form of desensitization of the βAR does not depend on phosphorylation of the receptor; rather it involves a conformational change in the receptor that sequesters the receptor from the cell surface, to the cell interior (Waldo et al., 1983; Lohse et al., 1990). Once the receptor is internalized, two normal functions of the receptor are lost: (1) the receptor no longer can bind agonists that are hydrophilic in nature and cannot pass through the membrane to associate with the receptor and (2) the receptor does not remain coupled to the Gs protein and can no longer stimulate adenylyl cyclase activity (Waldo et al., 1983). According to Waldo et al. (1983) internalization involves a change in the receptor to a vesicular form that may preclude a loss of the receptor from the cell.

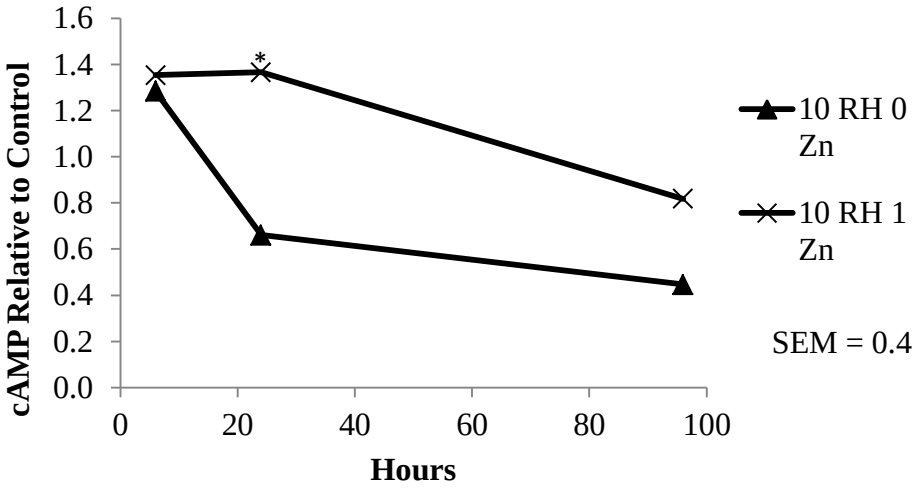
Another viable idea behind desensitization of the βAR is that the receptor goes through these three forms of desensitization in a step-by-step, sequential manner. The receptor is first phosphorylated by PKA, then by βARK and then is finally sequestered into the cell. Whereas others believed these processes have no chronologic specifics, Sibley et al. (1986) believes they build upon each other. According to Sibley et al. (1986), the receptor must first be phosphorylated and uncoupled from the Gs protein, after successful dissociation from the protein then the receptor can be internalized by the cell. Like others, the group believes that once the receptor is sequestered into the cell it is then internalized within a vesicle. Where Waldo et al. (1983) stated that this stage preludes a loss of the receptor from the cell, Sibley et al. (1986) found that while the receptor is within the vesicle, there is phosphatase activity that dephosphorylates the receptor. Once the receptor is dephosphorylated, the group then reported that the receptor is then transported back to the cell membrane where it can resume functioning.

Interestingly, each process can be blocked by using certain substrates in cell culture. At low β-agonist level, PKA induced phosphorylation causing uncoupling of the Gs protein can be blocked by the addition of a heat stable PKA inhibitor known as PKI (Lohse et al., 1990). Lohse et al. (1990) also found that phosphorylation of the receptor by βARK can also be inhibited by treating cells with heparin. Concanavalin A (ConA), a Kemptide consisting of Leu-Arg-Arg-Ala-Ser-Leu-Gly, was found to be a valid selective inhibitor of the internalization of the receptor caused by overexposure of the βAR to β-agonists (Lohse et al., 1990). These inhibitors have only been reported in cell culture studies.

Zinc (Zn2+) has been shown to have binding affinity to the β-adrenergic receptor. Instead of binding to the active site where agonists of the receptor bind to elicit intracrellular signals, Zn2+ binds to an allosteric, non-active site (Swaminath et al., 2002). When Zn2+ is added to cells in combination with a β-agonist, cAMP production is increased due to it causing higher affinity for the agonist and a decrease in affinity for the antagonist. It appears that there may be more than one area of the receptor that expresses interaction with Zn2+. A discovered binding site located at His-269 of the receptor was found to interact with Zn2+, and if this amino acid was removed, Zn2+ did not affect βAR cAMP production (Swaminath et al., 2003).

Our laboratory has conducted several cell culture experiments investigating the potential interaction of zinc and β-adrenergic receptor activity in cultured muscle satellite cells as measured by intracellular cAMP concentrations. In the first set of experiments, we concluded that added zinc and ractopamine HCl to culture media extended the cAMP production thus indicating that we reduced desensitization of the β-adrenergic receptor due to ractopamine in the presence of added zinc (Figure 1).

Figure 1. Effect of ractopamine hydrochloride (RH) and zinc (Zn) on cyclic AMP (cAMP) production in bovine satellite cells. Cells were administered RH and Zn after reaching 80% confluence. Treatments were provided along with 3% Horse Serum/Dulbeco's Modified Eagles Medium that contained 3% Antibiotic-Antimiotic and 0.3% Gentamycin. cAMP production was measured by use of an enzyme linked immunosorbent assay after 6, 24, and 96 hours of treatment which are represented by points on the graph. Standard error of the mean (SEM) represents the largest value. Points represented by an asterisk differ ($P < 0.05$) between treatments at a common time-point.



Interestingly, we did not observe the same biological effect with added zilpaterol HCl and zinc as compared to ractopamine. In fact, it appeared added zinc with zilpaterol HCl reduced cAMP concentrations compared to the zinc alone treatment at 6 h in culture (Table 1).

Table 1. Relative cAMP concentration[1] in bovine satellite cells treated with zinc (Zn) and zilpaterol hydrochloride (ZH).

	Treatment					
Hour	Control	10 µM ZH	1 µM Zn	10 µM ZH/ 1 µM Zn	SEM[2]	*P* – Value
00	0.228	0.233	0.225	0.225	0.009	0.857
06	0.336[ab]	0.322[b]	0.354[a]	0.327[b]	0.010	0.028
24	0.265	0.241	0.266	0.248	0.014	0.231
48	0.211	0.201	0.203	0.198	0.009	0.590
96	0.202	0.198	0.206	0.204	0.014	0.955

[a, b] Means in the same row having different superscripts are different, $P = 0.05$.
[1]Picomoles of cAMP/ml.
[2]Pooled standard error of the mean.

We have hypothesized that the ultimate binding affinity differences between ractopamine HCl and zilpaterol HCl and the β-adrenergic receptor may be an important determinant of zinc's ability to affect biological activity of the β-adrenergic receptor.

The mTOR Pathway

The mammalian target of rapamycin (mTOR) is a key regulator of growth in an animal dealing with nutrient designation and growth of tissues. mTOR is a member of a large superfamily of protein serine/threonine kinsases known as the PIK-related kinsases (Schalm and Blenis, 2002). The protein obtained its name after rapamycin was isolated from soil bacteria and administered to yeast cells that resulted in inhibition of TOR activity (Hay and Sonenberg, 2004). It was later determined that there are two TOR complexes: one that is sensitive to rapamycin (rapamycin-sensitive complex) and one that is not inhibited by rapamycin (rapamycin-insensitive complex) (Sarbassov et al., 2005). The growth characteristics associated with this kinase are affected by nutrient intake of the animal. Based on nutrient composition, mTOR effects include reorganization of the actin cytoskeleton, degradation of protein, membrane trafficking, PKC signaling, synthesis of ribosomes, transcription, autophagy, and can even cause cancer (Schmelzle and Hall, 2000; Sarbassov et al., 2005). Some of the reactions of mTOR based on available nutrients are known, however, much is still unknown about its activation and signaling.

The TOR proteins are high in molecular weight and are very conserved in amino acid structure. Mammailian TOR genomes encode for a protein that is up to 42% conserved with those found in yeast cells, with a high conservation of amino acids being found in the functional domains of the protein (Hay and Sonenberg, 2004). Not only is the TOR protein conserved, but other proteins within the cell that form complexes with it also do not vary much among species. These proteins, known as GβL, raptor (associated with rapamycin-sensitive complex), and rictor (associated with rapamycin-insensitive complex) all contain HEAT (for Huntignton, EF3, A subunit of PP2A, TOR1) and WD40 domains necessary for protein-to-protein interaction (Sarbassov et al., 2005; Hay and Sonenberg, 2004; Kim et al., 2002).

The exact mechanism of how rapamycin decreases mTOR effectiveness is not well understood. It has been shown that rapamycin binds to the FKBP12, which then binds to an allosteric site of the TOR protein far from the active site where raptor interacts (Sarbassov et al., 2005). This then decreases binding affinity of TOR to raptor and inhibits functionality of the complex. As discussed earlier, rapamycin-FKBP12 does not affect rictor-mTOR complexes. This is not well understood, but it is possible that rictor may inhibit rapamycin-FKBP12 interaction with mTOR.

It has been shown that cells that have been administered rapamycin, inducing the inhibition of the raptor-mTOR pathway, results in a large decrease in the size of the affected cells (Sarbassov et al., 2005). This poses the response that the mTOR pathway is indeed involved in cellular growth. The regulation of the mTOR pathway is still a partial mystery, however, free amino acids, growth factors, glucose, energy

status, many forms of stress, and insulin have been shown to cause regulation (Lynch et al., 2001; Schmelzle and Hall, 2000; Sarbassov et al., 2005). Translational effects of the mTOR pathway have been shown by the enhancement of activity of the p70s6k protein kinase and also by a reduction in activity of the 4E-BP1 (also known as PHAS-1), a protein responsible for inhibition of eIF4E (Lynch et al., 2001; Schmelzle and Hall, 2000). Under positive growth conditions, activation of p70s6k results in translation of 5' TOP (terminal oligopyrimidine tract) mRNAs, consequentially leading to an increase in ribosomal proteins and growth factors that up-regulate cell hypertrophy and even cell proliferation. (Schmelzle and Hall, 2000). If the mTOR pathway is inhibited by rapamycin, elF4E inhibition progresses and the cell cannot go through the G1 cell cycle, thus limiting cell proliferation (Schmelzle and Hall, 2000).

Both nutrition and growth factors can affect the mTOR pathway. A growth factor mediated effect involving mTOR is the activation of PI3 kinase (PI3K), the insulin receptor and insulin receptor substrates (IRS) (Schmelzle and Hall, 2000). PI3K initiates a growth response that is mediated by another protein known as PKB or AKT. According to Schmelzle and Hall (2000) some models show that this activation of PKB then increases mTOR activity while other plausible models propose that mTOR is the upstream regulator of PKB. This whole cascade is believed to be in response to insulin sensitivity of upstream mediators.

The mTOR Pathway and its Association with β-agonists and Zinc

The mTOR pathway, as mentioned earlier, is involved with nutrient regulated growth; therefore, it is of no surprise that β-adrenergic receptor agonists are associated with the pathway. Kline et al. (2007) performed studies involving rats that were provided clenbuterol, a β-adrenergic receptor agonist, and rapamycin in a 2x2 factorial. The group found that clenbuterol administrated rats showed an increase in phosphorylation of PKB, p70s6k, and ef4E binding protein. These are the same proteins that are involved with the mTOR mediated pathway discussed earlier. The rats that were given clenbuterol treatment showed increased growth (27-41%) and also showed a decrease in muscle atrophy. These results were nearly negated in rapamycin administered rats which showed 37 to 97% decrease in growth and a complete (100%) reduction in the muscle-sparing effect of clenbuterol. From these results, it can be concluded that clenbuterol effects are linked to growth-mediated responses of the mTOR pathway through activation of PKB.

Not only are β-agonists associated with mTOR but zinc also has been shown to play a role in activation of p70s6k. It was previously believed that zinc may aid in activation of p70s6k by involvement with PI3K and/or PKB, however, research performed by Trapolsi and Kimball (2001) evidenced that zinc may affect this pathway by directly regulating mTOR. The researchers found that phenanthroline, a heavy metal (including zinc) chelator inhibited amino acid and insulin activation of p70s6k by disallowing phosphorylation. The effects of the mTOR pathway were inhibited by reduced zinc abundance.

Effects of Zinc on Fiber Type

Zinc is a key component in major biochemical pathways, including transcription of DNA, translation of RNA, and cell division (Brown et al., 2002; Aggett and Comerford, 1995). Furthermore, Zn is an essential micronutrient in animal and human health (Salgueiro et al., 2000; Aggett and Comerford, 1995). In bovine satellite cell culture work, the addition of RH and Zn had no effect on MHC-I, MHC-IIA, MHC-IIX mRNA concentrations (Harris, 2013). Paulk et al. (2014) reported a linear decrease in the percent of MHC-IIA fibers as Zn concentration increased in pigs supplemented with RH and Zn. Also, a tendency was observed for an increase in the percentage of MHC-IIX fibers when supplemental Zn was fed in combination with RH (Paulk et al., 2014). While the effect of Zn on myosin isoforms has not been extensively researched, from the literature referenced we can conclude that Zn has very little effect on myosin isoforms. However, when Zn is fed in combination with a β-AA, Zn can alter the proportion of myosin isoforms in a muscle.

Our recent research has indicated that added zinc to the diet, as zinc methionine, 720 mg/hd/d, can alter fiber type in calf-fed Holsteins being fed zilpaterol HCl. In the *semimembranosus* muscle, zinc methionine (ZINPRO) fed cattle contained a greater abundance of MHC-I fibers as compared to control cattle which had a greater ($P < 0.05$) abundance of MHC-IIX. ZINPRO supplemented cattle had a greater percent of MHC-I fibers ($P < 0.05$; Table 2), and tended to have a higher percent of MCH-IIA fibers ($P < 0.10$; 47.01% vs. 47.83% respectively). Control cattle had a greater percentage of MHC-IIX fibers ($P < 0.05$; Table 2).

Table 2. Effect of zinc methionine in combination with zilpaterol hydrochloride on muscle fiber type composition in *semimembranosus* tissue.

	Treatment			
Item, %	Control	ZINPRO	SEM[1]	*P* - Value
MHC-I	11.86	12.92	0.026	0.001
MHC-IIA	47.01	47.83	0.017	0.069
MHC-IIX	41.13	39.25	0.017	0.001

[1]Pooled standard error of the mean.

CHROMIUM

Chromium is an essential nutrient in animal nutrition and for many years, typical rations for domesticated animals were thought to contain adequate levels of Cr. Over the years suggestions that Cr intake is generally low sparked interest in researching this element further. Preliminary studies displayed beneficial effects of Cr supplementation on biological function, health, and production of animals and humans. Some of the questions with Cr have been: Do traditional rations contain enough Cr in a bioavailable form? How much Cr should be supplemented? In what form should Cr be supplemented? Is it cost effective to supplement this micromineral? Chromium supplementation has been a popular topic in the swine industry for many years and more recently it is gaining popularity in the cattle feeding industry.

Chromium as a Supplement

Chromium was discovered by Louis-Nicholas Vauquelin in 1797. He was able to isolate chromium from chromium oxide by heating it in a charcoal oven in 1798. Chromium is a hard metal that exits in nature in oxidation states from Cr-2 to Cr+6, but the most abundant forms are Cr+3 and Cr+6 (Lukaski, 1999). Cr+6 would be easily reduced to Cr+3 in an acidic environment. As expected, the form of Cr will strongly impact its ability to be absorbed. Cr+6 is more readily absorbed than other forms like Cr+3. In humans, inorganic Cr+3 absorption is low (about 0.4 to 2 %), and has been inversely related to amount of Cr intake (Anderson and Kozlovsky, 1985; Bunker et al., 1984). Mertz and Schwarz (1955) observed that rats fed a Torula yeast diet were unable to efficiently remove glucose from circulating blood, but when foods rich in Cr were supplemented to their diet, this action was reversed. This observation resulted in the identification of a new dietary requirement called glucose tolerance factor (GTF). In 1957, GTF was proposed to be involved with maintaining normal blood glucose, prevents and cures impairment of glucose removal, and if deficient, the subject will become unable to effectively remove glucose from circulation (Schwarz and Mertz, 1957). In 1959 Cr3+ was identified as the active ingredient of the GTF (Lukaski, 1999). Glucose tolerance factor was thought to consist of chromium(III), cysteine, nicotinic acid, glutamic acid, and glycine (Toepfer et al. 1977). There was a rapid decline in GTF research in the mid 1980's after Mertz and coworkers were unsuccessful in isolating Brewer's yeast GTF and unsuccessful in attempts to synthesize or characterize synthetic GTF after 20 years of research (Toepfer et al., 1977). More recently Sun et al. (2000) and Vincent (2000) claimed that Cr modifies glucose metabolism through an oligopeptide known as chromadulin. Chromadulin consists of glycine, cysteine, aspartate, and glutamate and binds with high affinity to four chromic ions (Vincent, 2000, 2001). This enables Cr to be involved in the autoamplification of insulin signaling to maintain the active conformation of insulin receptors, and cause greater glucose to enter insulin-sensitive cells (Vincent, 2000 and 2001).

To describe the mode of action in more detail, when circulating glucose concentrations rise insulin is secreted in the pancreas and released into the blood stream. Insulin will bind to the insulin receptor on the outside of the cell membrane. Although not certain, it is believed that insulin binding to the outside of the receptor causes Cr to enter the cell through iron-transport protein, transferrin (Vincent 2001). Once Cr is inside the cell it will bind with apochromodulin to create holochromodulin (chromodulin). Chromodulin will bind to the inside of the insulin receptor which will increase the stability of the insulin receptor and increase the binding affinity of insulin to its receptor. This binding affinity starts a cascade of phosphorylation events that ultimately produces protein kinase B. Protein kinase B signals Glut-4 transporters to move from storing vesicle within the cell to the cell membrane. At the cell membrane Glut-4 transports allow glucose to cross the cell membrane and enter the cell. As circulating glucose concentrations drop insulin secretion will cease and eventually insulin will not be bound to the insulin receptor anymore. This causes chromodulin to release from the insulin receptor and ultimately stops the production of protein kinase B. This will cause the Glut-4 transporters to leave the cell membrane and return to a Glut-4 containing vesicle within intracellular fluid.

There are many organic and inorganic sources of Cr that have been the subject of research over the past several decades. One organic source of Cr is a high-Cr-yeast (CrY). Other organic sources would include Cr-methionine (CrMet), Cr-picolinate (CrPic), Cr-nicotinic acid complex (CrNic), chelated-Cr (CCr), and Cr-propionate (CrPro). An inorganic form that has been researched is Cr-chloride ($CrCl_3$). The actual bioavailability of these different sources is still relatively unknown, but generally organic sources have displayed more advantages in production, immune response, and blood metabolites, suggesting they are more bioavailable.

Even with extensive research concerning the mode of action and supplementation to ruminants, sufficient data to determine a Cr requirement for beef cattle is still lacking. In their 1980 publication, the National Research Council (NRC) estimated the maximum tolerable concentration of trivalent chromium, as $CrCl_3$, to be 1,000 mg Cr/kg diet. No negative effects were reported in steers fed 4.0 mg Cr-polynicotinate complex/kg diet for 70 d (Claeys and Spears, unpublished data). Chromium in the trivalent form is much less toxic than Hexavalent chromium (NRC, 1980).

Effect of Chromium on Glucose Metabolism Following a Glucose Tolerance Test (GTT)

Supplementation of microminerals will often yield varying results, but chromium's effect on glucose metabolism has been more consistent than its effect on performance. Organic sources of Cr, such as CrPro or CrMet have been some of the more predictable sources (Kegley et al., 2000; McNamara and Valdez, 2005).

High-Cr-Yeast. No differences were determined in glucose or insulin concentrations, glucose clearance rate (k), or insulin sensitivity when supplementing 0 or 0.4 mg/kg of CrY (Kegley and Spears, 1995).

Cr-nicotonic acid complex. Kegley et al. (1995) reported no difference in basal glucose concentrations when supplementing 0 or 0.4 mg/kg of CrNic. There was a significant time x dietary treatment interaction on plasma glucose concentrations post-infusion, in that steers supplemented with 0.4 mg/kg of CrNic displayed increased glucose concentrations at 15 m post-infusion and no differences between treatments by 30 m post-infusion (Kegley and Spears, 1995). Chromium also increased the k following a GTT (Kegley and Spears, 1995). Insulin concentrations following a GTT were increased for CrNic supplemented cattle. Kegley et al. (1997) found that CrNic supplementation did not affect glucose or insulin concentration, k, glucose half-life (T½), or insulin to glucose ratio (I:G).

Cr-picolinate. Bunting et al. (1994) reported no difference in basal glucose concentrations when supplementing 0 or 0.37 mg/kg of CrPic. There were significant improvements in k, T½, and area under the glucose curve (AUC) for steers and heifers supplemented with CrPic (Bunting et al., 1994). No

differences were detected between treatments for insulin concentrations or AUC (Bunting et al., 1994). This, combine with the faster k concluded that the CrPic-supplemented cattle were more sensitive to insulin. Chromium-picolinate supplemented to lambs resulted in no differences between treatments for mean glucose or insulin concentrations pre- or post-infusion (Gentry et al., 1999). However, from 15 to 30 m post-infusion CrPic-supplemented lambs did remove glucose from circulation more rapidly (Gentry et al., 1999). Following a GTT, sheep supplemented CrPic for two weeks produced greater insulin and lower glucose concentrations than control steers (Kitchalong et al., 1995). No differences were detected during a GTT at ten weeks (Kitchalong et al., 1995).

Cr-methionine. Kegley et al. (2000) reported a significant time x dietary treatment on plasma glucose concentrations post-infusion. Plasma glucose concentrations of calves supplemented with 0.4 mg/kg of CrMet were lower than calves supplemented with 0.8 mg/kg of CrMet and non-supplemented calves at 5 and 10 m post-infusion, but no differences were reported by 15 m post-infusion. Kegley et al. (2000) concluded no differences between treatments in k or T½ when supplementing 0, 0.4, or 0.8 mg/kg of CrMet. Insulin concentrations following a GTT were increased from cattle supplemented with CrMet (Kegley et al., 2000), causing a reduction in insulin sensitivity.

Cr-Propionate. Sumner et al. (2007) reported that basal glucose levels were increased when supplementing growing Holstein heifers with 10 and 15 mg/d of CrPro. It was also concluded that supplemental CrPro increases the k and T½ following a GTT (Sumners et al., 2007). Sumner et al. (2007) saw no overall effects of CrPro on insulin concentration following a GTT. Chromium-propionate supplemented to heifers displayed a 36% reduction in AUC (Sumners et al., 2007). As a result of the increased k and no changes in insulin concentrations, CrPro supplementation increased insulin sensitivity (Sumners et al., 2007).

CrCl3. Kegley et al. (1995) reported no difference in glucose or insulin concentrations, k, or insulin sensitivity when supplementing 0 or 0.4 mg/kg of CrCl3. Two years later, Kegley et al. (1997) exhibited that CrCl3 did not affect glucose concentrations or k, but insulin concentrations following the GTT were lower from 10 to 25 m for CrCl3-supplemented steers. This suggests that cattle were more sensitive to insulin within the first 25 m post-infusion.

Effect of Chromium on Glucose Metabolism Following an Insulin Sensitivity Test (IST)

Cr-nicotonic acid complex. Prior to infusion, Con cattle had lower plasma glucose concentrations than cattle supplemented with CrNic (Kegley et al., 1997). Post-infusion there was a time by treatment interaction, in that cattle supplemented with CrNic had lower glucose concentrations from 45 to 180 m than Con cattle (Kegley et al., 1997). Supplemental CrNic had no effect on insulin concentrations pre- or post-infusion (Kegley et al., 1997). Kegley et al. (1997) suggest that CrNic-supplemented cattle were either more sensitive to insulin or the insulin had a longer lasting effect.

Cr-picolinate. Bunting et al. (1994) concluded no differences in mean glucose concentrations pre- or post-infusion in steers supplemented with CrPic. No differences in k and T½ in steers, but Bunting et al. (1994) did see improvements in k and T½ of heifers supplemented with CrPic. Cattle supplemented with CrPic had no difference in mean insulin concentrations pre- or post-infusion, and insulin concentrations returned to baseline concentrations by 90 m post-infusion (Bunting et al., 1994). Supplemental CrPic had no effect on I:G (Bunting et al., 1994). When supplementing CrPic, Kitchalong et al. (1995) concluded no differences in mean glucose concentrations pre- or post-infusion, k, or T½ in lambs. Sheep supplemented with CrPic had no differences in mean insulin levels pre- or post-infusion, and insulin levels returned to baseline concentrations by 90 m post-infusion (Kitchalong et al., 1995). Additionally, supplemental CrPic had no effect on I:G (Kitchalong et al., 1995).

Cr-methionine. Kegley et al. (2000) detailed that steers supplemented with CrMet had lower glucose concentrations pre- and post-infusion. Kegley et al. (2000) discovered an increase in k from 5 to 10 m

post-infusion in CrMet-supplemented cattle, but no differences were found from 10 to15 or 15 to 30 m post-infusion. Chromium-methionine tended to decrease the T½ of steers from 5 to 10 and 10 to 15 m post-infusion (Kegley et al., 2000). Kegley et al. (2000) reported no differences between treatments pre-infusion, but post-infusion, a time x treatment interaction concluded that steers supplemented with CrMet had increased insulin concentrations at 5 and 15 m. Chromium-methionine caused an increase in I:G concentrations at 5 and 15 m post-infusion, but not at any other time point (Kegley et al., 2000).
CrCl3. Prior to the infusion, Con cattle had lower plasma glucose concentrations than cattle supplemented with CrCl3 (Kegley et al., 1997). Additionally, post-infusion there was a time by treatment interaction, in that cattle supplemented with CrCl3 had greater decrease in glucose concentrations from 15 to 180 m than Con cattle. Supplemental CrCl3 had no effect on insulin concentrations pre- or post-infusion (Kegley et al., 1997). Overall, Kegley and colleagues suggested that CrCl3-supplemented cattle were either more sensitive to insulin or the insulin had a longer lasting effect (1997).

Effect of Chromium on Lipid Metabolism

During a GTT. Kitchalong et al. (1995), Gentry et al. (1999), and Sumner et al. (2007) have reported a decrease in mean NEFA levels when supplementing chromium (CrPic, CrPic, and CrPro, respectively). In beef steers, Kegley et al. (2000) reported no difference in NEFA concentrations when supplementing CrMet. Additionally, Bunting et al. (1994) concluded no difference in NEFA concentrations when supplying CrPic to growing Holstein calves.

During a IST. When supplementing CrPic, Kitchalong et al. (1995), concluded that Cr supplementation causes decreases in circulating NEFA levels (pre- and post-infusion); however, Bunting et al. (1994) did not notice any significant differences in NEFA concentrations between treatments.

Baseline (no metabolic challenge applied). Uyanik (2001) concluded that Cr supplementation for (CrCl3) for 55 d resulted in lower circulating triglyceride concentrations. Besong (1996) and Hayirli et al. (2001) both reported that Cr (CrPic and CrMet, respectively) decrease circulating NEFA concentration prepartum in dairy cows but no differences were determined postpartum. Chromium-picolinate reduced NEFA concentrations in Angus and Simmental cows 2 to 5 years of age, but not in older cows. Depew et al. (1998) concluded that CrPic had an age x treatment interaction on circulating NEFA concentrations when measured in dairy calves prior to feeding, but post-feeding measurements displayed that CrPic supplementation reduced circulating NEFA concentrations. When supplementing CrPro to dairy cattle, McNamara and Valdez (2005) concluded that Cr supplementation increased the net synthesis of fat in adipose tissue, while reducing the net release of fat.

Our recent cell culture data indicated that a time-dependent treatment of chromium propionate (CrPro) increased GLUT4 levels in IM adipocyte cultures. This GLUT4 data indicated that treatment of CrPro may be regulating the early phase of IM adipocyte differentiation. The level of GLUT4 was steadily increased in both IM andSC adipocytes during the late phase of adipocyte differentiation. However, bovine satellite cells treated with chromium propionate in this study showed no effect on GLUT4 mRNA expression and had decreased GLUT4/GAPDH protein ratio in a dose-dependent fashion. The variation in these results between adipocytes and muscle satellite cells indicated that CrPro has differential effects on different tissues. In essence, the CrPro decreased GLUT4 protein levels in muscle cell cultures (Figure 2) indicating those cells have increased efficiency of glucose uptake due to exposure to increased levels of CrPro. In contrast, each of the two adipogenic lines had opposing responses to the CrPro. It appeared that CrPro had the most stimulative effect of GLUT4 response in the IM adipocytes as compared to SC adipocytes (Figure 2). This suggests opportunities to potentially augment marbling in beef cattle fed elevated levels of CrPro during the finishing period. In addition, skeletal muscle growth could be further enhanced in cattle being fed both a beta-adrenergic agonist and CrPro the last 20 to 40 days on feed.

Figure 2. Differential effects of added chromium propionate on cultured adipocytes and muscle cells (Tokach, R. J., 2011).

Adipocytes	IM	SC	Muscle Cells	
AMPK-α	+	-	Cr	Increases myotube size ↑
GLUT 4	+	-	Cr	Number of myotubes ↑
PPAR-γ	+	-	GLUT4	No change
Lipid droplets	↑↑	↑–	GLUT4/GAPDH	↓

CONCLUSIONS

Numerous research trials have been conducted the past 20 years to determine the usefulness of supplementing micronutrients. The beef industry still lacks knowledge regarding the appropriate timing and stage of production for which supplementation of micronutrients would be the most cost effective while maintaining the benefits to animal health and performance. Additionally, the exact mechanism and mode of action for these supplements is not completely understood. Furthermore, the effect of these supplements on other factors which influence beef production, such as the secretion of hormones, remains relatively unknown.

Research investigating zinc and chromium has revealed inconsistent results from trial to trial. These variations in results are attributed to disparities in Cr status of the cattle prior to initiation of the study, basal Zn and Cr level in the diet, level of Zn and Cr supplementation, and most importantly, the source of Zn and Cr supplementation (Spears, 2000). Metabolic variations noted in research trials can result from the four reasons previously listed and variations in the amount of product infused during a metabolic challenge.

It is well known that in the modern beef industry, growth promoting agents such as implants and β-adrenergic agonists will continue to play a crucial role in efficient beef production. Though performance improvements have been made, the current beef industry is losing approximately a billion dollars each year to health related issues. Complimenting implants and β-adrenergic agonists and reducing cost associated with health are the two areas of cattle feeding that could benefit the most from supplementation of micronutrients, like Zn and Cr. There are some indications that Cr could potentially influence the marbling potential of beef cattle, which could improve the quality of beef when complimenting implants and β-adrenergic agonists. Chromium and zinc could also definitely play a role in improving the health of feed cattle and recovery rates if exposed to an immune challenge. Chromium and zinc are a very low-cost method to potentially decrease health risks, while markedly improving feedlot performance.

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Feeding To Maximize Milk Protein

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SUMMARY

- Yield of milk protein is an economical important factor as more protein is needed to produce cheese (over 40 percent of milk consumed in the U.S. as cheese) and consumer interest in Greek yogurt higher in protein content.
- Increasing milk protein is impacted by genetics, microbial amino acid synthesis, and feed sources (rumen undegraded protein or RUP).
- Dairy farmers are paid for true protein content (casein and whey protein fractions, not milk urea nitrogen which is 0.2 percentage point lower than total or crude protein content).
- Nutritionists and dairy farmers must provide the optimal amount and balance of amino acids to the mammary gland for optimal milk protein synthesis.

ECONOMICS OF MILK COMPONENTS

Most U.S. milk markets price milk based on the value of a pound of milkfat, true milk protein, and other milk solids. Table 1 reflects the U.S. value over the last year. Dairy farmers challenge is obtain the maximum level of milk protein based on the genetic value of their herd leading to improvement milk income.

Table 1. Value of milk components in the 2014 in the U.S.

Component	January	March	June	November
	---------- Dollars per pound ----			
Milk protein (true)	3.54	4.51	3.34	3.90
Milk fat	1.78	2.04	2.44	2.20
Other solids	0.38	0.47	0.49	0.45

An economic comparison is the value of increasing milk components by 0.1 point based on an average of 70 pounds of milk and using November, 2014 milk component prices from Table 1. For each one point increase in milk protein (from 3.0 to 3.1 percentage points for example), the increase in milk value is worth an extra 27 cents per cow per day. For each point increase in milk fat (from 3.6 to 3.7 percent for example), the increase in milk value an extra 15 cents per cow per day.

EVALUATING MILK COMPONENTS

One guideline in evaluating milk components is to determine if the herd is normal (at or above breed average). Each year Hoard's Dairyman Magazine publishes breed averages based on DHIR values (Table 2).

Table 2. Typical milk components for varies dairy breeds in 2014 (Hoards Dairyman Magazine).

Breed	Milk fat	True milk protein	Ratio (protein/fat)
	---------- % ----------		
Ayrshire	3.87	3.16	82
Brown Swiss	4.03	3.31	82
Guernsey	4.53	3.31	73
Holstein	3.73	3.02	81
Jersey	4.83	3.64	75

To evaluate milk components in your herd, compare milk protein percentage to milk fat percentage reviewing ratios and levels in various groups using DHI records or electronic summaries.

- Milk protein to milk fat ratio of the entire herd by month
- Ratio in various pens (fresh, high cows, heifers, and other pens)
- Ratio based on days in milk (< 100, 100 to 200, and over 200 days in milk)
- Ratio based on lactation number (1^{st}, 2^{nd}, and 3^{rd})
- Changes over the last one to three years
- Ratio in cows less than 50 days in milk reflecting excessive body weight loss and metabolic risks
    - Milk protein: fat ratio below 70%
    - Milk fat tests one full point over the herd average

GENETIC CONSIDERATIONS

Heritability of milk components is high with milk fat percent at 0.58, milk protein percent at 0.49, and lactose percent at 0.55. The heritability of milk yield is lower at 0.27. Other genetic relationships are listed below as correlations.

Correlation between percent fat and percent protein	+ 0.45 to + 0.55
Correlation between percent fat and solids-not-fat percent	+ 0.40
Correlation between percent protein and solids-not-fat percent	+ 0.81
Correlation between milk yield and milk fat percent	- 0.15 to – 0.30
Correlation between milk yield and milk protein percent	- 0.10 to – 0.30

A positive correlation indicates that as you select for one trait, the other trait will increase in the same direction. For example, if you select for milk fat percent, milk protein percent should also increase. Dairy managers must realize if selection for higher milk yield is implemented, milk components could decline. The value of milk is based on pounds of milk fat and milk protein, not percentage. Selection based on pounds of milk fat and/or milk protein results in the greatest economic improvement. Some managers will base selection on cheese yield (includes the economics of both components in an index).

OPTIMIZING MILK PROTEIN

If milk protein yield and/or percent are low, evaluate sources of amino acids needed by the mammary gland to synthesize milk protein (casein). Sources of amino acids for milk protein include microbial synthesis (over 60 percent of the total amino acids needed), dietary sources (rumen undegraded protein or RUP), and mobilized animal tissue (limited source). Strategies to optimize amino acid production for dairy cows are listed below:

- Maximize microbial protein synthesis and passage to the small intestine. Microbial amino acid yield is related to organic dry matter digestibility.

- Optimize feed and energy intake (drives microbial growth)
  - Adequate physically effective fiber reducing rumen acidosis
  - Provide 24 to 28 percent total starch in the ration
  - Add 3 to 5 percent sugar (total of 5 to 7 percent)
  - Evaluate the level of rumen fermentable carbohydrates with a target level over 40 percent (add the percent starch, sugar, and rumen digestible fiber).
- Feed digestible RUP sources and blend different RUP sources to balance amino acid composition and reduce feed variability.
  - Lysine sources include blood meal and heat treated soybean products
  - Methionine sources include corn by-products and fish meal.
- Consider the initial pound of protein supplement from soybean, canola, or cottonseed meal as a source of peptides and amino acids for rumen microbes.
- Add a source of rumen protected amino acids as indicated by an amino acid model (such as Dairy NRC 2001, CPM, AMTS, Spartan III, Nittany Cow, DNS, or other model systems).

Table 3. Target formulation levels for percent of lysine and methionine in metabolizable protein (Whitehouse et. al, 2009)

Model	Lysine	Methionine
NRC	6.60	2.28
CPM	6.38	2.24
AMTS/DNS	6.38	2.24

- Lysine and methionine are considered as first limiting amino acids with histidine possible in grass-based forage program.
  - Lysine levels should range from 6.2 to 6.6 percent
  - Methionine levels should be range from 2 to 2.2 percent
  - Ratio of lysine to methionine should be 3:1
  - If protected amino acids are supplemented, milk production and/or milk components can respond within two weeks
- Insure metabolizable protein requirements of close up dry cows are met.
- Excessive amino acids can be used as a source of glucose which is not economical.

Use of an amino acid model is recommended to dairy managers that have accurate dry matter intake (adjusted for weigh backs), conduct routinely forage test, and achieve high milk protein production (over 2.5 pounds of true protein per cow per day). Table 4 illustrates that dairy managers must manage nutrients from the rumen, and small intestine to provide precursors needed by the mammary gland to synthesis milk yield and component..

Table 4. Relationships between products of digestion and changes in milk yield and components (based on controlled research studies).

Products	Response (compared to control cows)		
	Milk yield	Fat	Protein
Rumen VFA			
Acetate	++	++	-
Propionate	-	---	++
Butyrate	++	+++	+
Small intestine			
Glucose	++	---	-
Amino acids	++	--	++
Fatty acids	+	+++	na

Key: + small positive response, +++ large positive response, - small negative response, --- large negative response

USING MILK UREA NITROGEN (MUN)

Milk processing plants and milk testing programs can provide dairy managers with milk urea nitrogen (MUN) values on bulk milk and individual cow samples. MUN is a useful tool that can allow dairy managers to monitor feeding, environment, and management changes in their herds.

When cows consume feed containing protein, part of the protein is degraded to ammonia by rumen microbes (rumen degraded protein or RDP). If bacteria cannot capture the ammonia converting it over to microbial protein, excess ammonia is absorbed across the rumen wall. Because ammonia can shift blood pH, the liver converts ammonia to BUN to be excreted or recycled. Because milk is synthesized from blood and BUN values are elevated, MUN values will also be higher. MUN represents about 0.19 percentage point of the total 3.2 percent total protein in Holstein milk.

Herds can have a different optimal level depending on the time of feeding relative to milking time, feeding total mixed rations (TMR) compared to component-fed herds, pasture-based compared to stored feeds, cow eating patterns during the day, and heat stress. The value in using individual cow MUN tests is to monitor changes in MUN values within a herd and groups of cows. Milk plant MUN values will vary due to machine standards and sampling differences.

- Develop a MUN baseline that is "normal" for your herd (values may range from 8 to 16 with most herds from 10 to 14 milligrams per deciliter). Illinois recommendations are 8.0 to 12 mg/dl.
- When the MUN value changes by more than 2 to 3 points (normal variation), look for changes in your herd that caused this MUN shift.
- Looks at weekly averages in your MUN values from your milk processing plant as variation occurs from day to day.
- Review DHI values to compare differences in MUN related to milk yield, lactation number, feeding groups, and stage of lactation.

The challenge is for rumen microbes can capture rumen ammonia as microbial protein. Feeding excessive total crude protein in the ration resulting in excessive ammonia being wasted. Too much rumen degraded protein (RDP) and/or soluble protein can raise MUN even if the total crude protein level in the ration is optimal. If rumen acidosis occurs, microbial protein growth will be slowed and ammonia not captured. Rations low in rumen fermentable carbohydrate (such as starch, sugar, and/or digestible fiber) can reduce microbial growth leading to higher MUN values. New crop corn silage may not have the same

level of rumen fermentable starch (less starch is available in the rumen until three to four months after ensiling). Shifting from processed corn silage to unprocessed or improper processed corn silage reduces rumen fermentable starch. Cows consuming lush pasture can consume excess total protein and RDP. Processing / grinding cereal grain improves the rate of fermentation in the rumen and ammonia capture while avoiding rumen acidosis.

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The Future for Protected Fats in Dairy Nutrition

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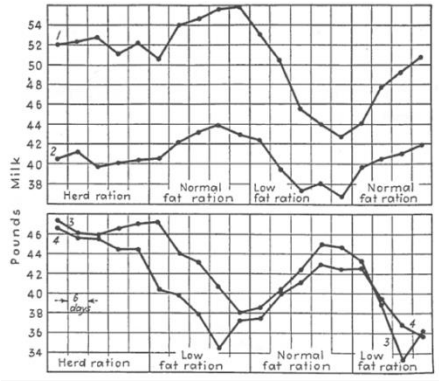
SUMMARY

- The history of using fats in dairy diets is reviewed briefly
- High producing cows require supplemental energy from fat to achieve adequate energy intake
- It is essential to include adequate forage in diets containing fat
- Including fat in diets increases flexibility of diet formulation
- Development of various rumen-inert fats has further increased options for dairy nutritionists
- Opportunities for using fat for improved reproduction, increased milk fat percent or modified milk fat composition are highlighted
- As milk yield of cows increases, use of increased amounts of rumen-inert fats is assured

INTRODUCTION

Any discussion of the future requires first to orient the reader by discussing the past and present. So I will begin this story with the beginning of modern research on exploring use of fat in dairy cow rations. Detailed studies of fat utilization by rats and cows were carried out from 1928 to 1945 by L.A. Maynard and colleagues. These were published mainly in the Journal of Nutrition and in Experiment Station bulletins. Of particular note, Maynard commented in his first edition of *Animal Nutrition* (1937, page 418) that cows consuming less fat in the diet than produced in the milk tended to produce less milk than those fed more fat, and "in other experiments, no certain lowering (of yield) occurred where the fat content of the ration was not reduced below the amount secreted in the milk" (**Figure 1**). I began studying fat utilization and metabolism in lactating cows in 1965, and by 1990 I had rediscovered Maynard´s observation!

Figure 1. Milk yield is normal on a herd ration or a normal fat ration. Yield decreases when a low fat ration is fed. (Maynard, 1937).



NRC Dairy and Fat

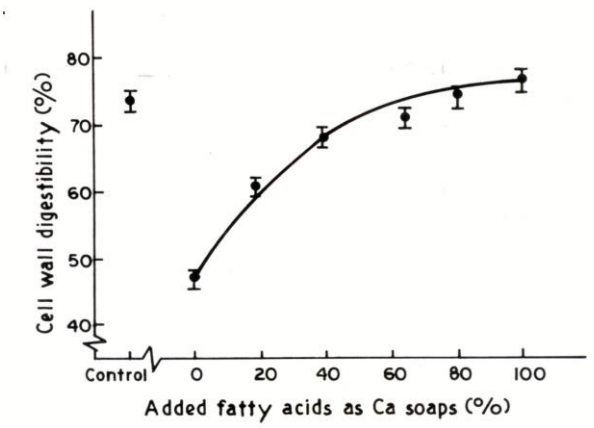
Maynard´s longtime colleague, J. K. Loosli, was chair of the first four editions (1945, 1956, 1966, 1971) of the NRC Nutrient Requirements of Dairy Cattle; none of these mentions fat as a component of rations for calves or cattle. In the 5th revised edition (1978) dietary fat is acknowledged to be needed by young

calves; for cows it is mentioned as a "special aspect of dairy nutrition", and that adequate fat is provided by rumen microbial synthesis, but supplemental fat has negative rumen effects and is too expensive to be included in dairy rations. The 5th edition did cite our work that fat could be used to avoid excessive use of grain while assuring adequate energy and forage intake in order to maintain milk fat percent, and that 5% fat in the ration is acceptable in some feeding circumstances. However, no information on fat content of feedstuffs is provided. In the 6th revised edition (1989) dietary fat is still discussed as a special aspect of dairy nutrition; also need for adequate calcium intake is noted, citing our work (Palmquist and Conrad, 1980). For the first time, fat (ether extract) content of feedstuffs is included in feed tables. At that time I was advocating that fat in dairy rations should be expressed as fatty acid content, not ether extract; though forages have quite low concentrations of fatty acids (~2% of dry matter), ether extract content can be considerably greater, caused by presence of ether-soluble non fatty-acid matter. This is an important consideration because forages compose a major part of dairy rations. The last (2000) revised edition of dairy NRC addresses fat in dairy diets in considerable detail, both as an important source of energy as well as in a separate chapter of its own, covering aspects of fat metabolism and use in feeds.

Progress in Fat Research

Considerable research on fat metabolism in dairy cows was carried out in the 1960´-70´s in the UK by Moore, Storry and others. These studies, though certainly useful, usually examined effects of individual fatty acids on milk fat composition, used small numbers of low-producing cows, and provided little information on diets, intake or statistical analysis. By the 1970´s, some tallow and animal vegetable blends of fat were being incorporated into high energy commercial dairy concentrate feeds, and whole cottonseed was being used in total mixed rations, primarily in the Southwest. For the most part, however, adding fat to dairy rations was not widely practiced. We (Palmquist and Jenkins, 1980) published a review on fat in lactation rations that more or less established the status and issues needing to be addressed with regard to usefulness of fat for lactating cows. In order to really know the usefulness of fat for lactation, the rumen metabolism issues were of primary importance. We had established that fiber and calcium intakes were critical when fat was added to diets. Focusing on that, we discovered that providing fat as the insoluble calcium salts of fatty acids completely overcame the issue of fatty acid inhibition of fiber digestion (**Figure 2**). Following development of patents for commercialization of manufactured calcium soaps, it remained to be established the conditions for proper use of the product in lactation rations. An alternative product, hydrogenated fatty acids (mainly palmitic and stearic, with ~15% oleic acid) was gaining popularity at the same time, so many research trials included both, resulting in a great number of published papers and rapid progress in knowledge of using rumen-inert fats in dairy rations and their application in the field. At the same time, non-rumen inert fats, such as cottonseed and whole roasted soybeans were being used more widely. An excellent summary of the non-energy effects of fats in dairy rations was presented by Tom Jenkins at this conference (Jenkins, 2002).

Figure 2. In vitro cell wall digestibility increases as the proportion of fatty acids as calcium soaps increases. (Palmquist and Jenkins, 1982).

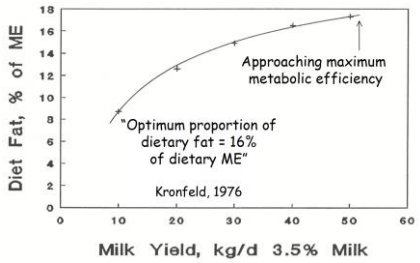


GUIDELINES FOR INCLUDING FAT IN LACTATION RATIONS

How Much Fat to Feed?

As fat became more widely used, questions arose as to what is the right amount to be used in feeding dairy cows. Much of the early work was done with low producing cows that had no need for a high energy diet. In other studies, too much fat was fed, with negative effects on milk yield. We had been studying the metabolism of fats, focusing on how fat was used in the animal. We showed that by far the greatest amount of absorbed fat is used directly for milk fat synthesis; relatively little is used for oxidative energy in well fed cows, and deposition in body tissues depends on stage of lactation and energy balance (Palmquist, 1994). Putting this information together with newer information on digestibility, we concluded that the right amount of fat to feed is the amount that is produced in milk, an observation made by Maynard some 5 decades earlier. One can extrapolate this information to calculate the proportion of NEL in the diet provided by fat (**Figure 3**). It can be seen that as the proportion of fat in the diet increases, efficiency increases curvilinearly, approaching a maximum asymptote near 16% of NEL as fat; similar to a maximal theoretical efficiency predicted by Kronfeld (1976) from known efficiencies of metabolic pathways. This information is useful especially when formulating dairy rations in hot weather conditions.

Figure 3. Relation between proportion of fat ME in diet dry matter and milk yield when dietary fat is fed in an amount equal to milk fat yield (Palmquist, 1992).



Fat and Dry Matter Intake

Including fat in the ration may decrease total dry matter intake; whereas relatively small effects are observed when supplementing saturated fatty acids, effects are greater with supplementing unsaturated fats (Bremmer et al, 1998; Allen, 2000). That latter is true with feeding calcium salts of palm fatty acid distillate, which contains on average 35% oleic acid (18:1) and 6–8% linoleic acid (18:2). Calcium salts are partially biohydrogenated in the rumen, extent depending on particle size and rumen conditions, but usually half of the unsaturated fatty acids will pass to the intestine (Wu et al., 1991), as compared to 85–90% biohydrogenation of usaturated dietary fats generally (Jenkins et al., 2008). The effect on intake seems to be strongly tied to total unsaturation of absorbed fatty acids. The role of fat in regulating feed intake is likely mediated by their oxidation in the liver (Allen, 2014); stearic acid is not taken up by the liver (Bell, 1980), explaining the smaller effect of more saturated fatty acids. The effect is also dependent on the physiological and energy balance state of the animal; when the oxidation of acetyl Co-A in the liver is low, the likelihood of fatty acid oxidation in the liver inhibiting feed intake is decreased (Allen, 2014). A key to minimizing effects of unsaturated fatty acids on intake is to assure that fat is not fed to excess. Absorbed fat is delivered to the general circulation via the portal vein, so that liver is not the first organ it encounters. For example, we observed no differences in DMI of cows supplemented with tallow or whole roasted soybeans, even though absorption of linoleic acid was more than double for those fed whole roasted soybeans (Morales, et al., 2000a,b). In that study, cows consumed fat in amounts similar to that produced in the milk and secreted much of the absorbed unsaturated fatty acids directly into the milk.

While dietary fat provides energy and increases efficiency of milk synthesis, it does not provide energy for synthesis of microbial protein, a factor that must be considered in formulation diets. However, in many studies (Stern et al., 1994), feeding supplemental fat did not reduce flow of microbial protein from the rumen.

Fat and Forages

As noted above, supplementing fat permits greater amounts of forage to be fed; as well, it is critical to feed sufficient forage to minimize negative effects of supplemental fat in the rumen. As fatty acids enter the rumen, either as fed directly or by lipolysis, they become associated with the surfaces of forage particles (Harfoot et al., 1974). We have found that alfalfa absorbs a much greater amount of fatty acid than does grass or corn silage (Palmquist and Yang, 1999); Smith et al. (1993) reported that milk yield was greater when cows fed diets containing tallow or whole cottonseed included alfalfa, compared with corn silage. Onetti and Grummer (2004), in a meta analysis of diets containing tallow, saturated fatty acids or calcium soaps, reported that different responses to supplemental fats were observed depending on the main forage in the basal diet. Milk yield increased when tallow diets included alfalfa, whereas yield response to calcium soaps was positive only with corn silage as forage. Milk response to feeding hydrogenated tallow fatty acids was independent of forage source. The authors concluded that "Interactions between fat type and characteristics of the basal diets must be identified in order to predict the production responses of dairy cows to supplemental dietary fats."

At today´s levels of milk production, supplemental fat is required in lactation rations. Diets containing only cereals and forages provide no more than 3% of dry matter as fatty acids. Tallow and yellow grease generally are not desirable supplements; however, DePeters and colleagues (DePeters et al., 1987, 1989; Avila et al., 2000) have reported excellent responses with these when included with high quality West Coast alfalfa. More desirable in most rations are whole cottonseed or whole roasted soybeans; these can provide 2% of ration dry matter in most feeding situations. Above 5% of dietary fatty acids, rumen-inert sources are recommended, especially if forage is limited in amount or quality. For meta analysis of responses to various fat supplements, see Rabiee et al. (2012).

When to Feed Fat?

There is some debate as to whether feeding fat in early lactation is desirable. It would seem to be desirable to feed when cows are in negative energy balance; however, as noted above, cows have limited ability to metabolize fat, and one must include mobilized body fat in the equation. Therefore, adding fat to rations before 6 weeks of lactation must be done with caution. Increasing protein supply to maximize milk yield increases fat secretion into milk, so may minimize the problem (Palmquist and Weiss, 1994). Many believe also that added fat is not needed past mid-lactation. In our experience, using supplemental fat in place of grain past peak milk yield maintains milk and fat yield without getting cows too fat; these responses are likely mediated by insulin.

SPECIAL CONSIDERATIONS FOR INCUDING FAT IN LACTATION RATIONS

Fat and Reproduction

The extensive and in-depth research on the role of supplemental fats for reproduction in dairy cows by Thatcher and colleagues at Florida has demonstrated unique roles for specific fatty acids at different stages of lactation. In a recent study (Silvestre et al., 2011) supplemented (1.5% of dry matter) cows in a large commercial herd with calcium salts of palm oil or safflower oil (high linoleic acid, n-6) from 30 days prepartum to 35 days postpartum. From 35 to 160 days postpartum they were supplemented (1.5% of dry matter) with calcium salts of palm oil or fish oil (n-3 fatty acids). The pro-inflammatory state induced by feeding n-6 fatty acids enhanced numerous aspects of neutrophil physiology and augmented a greater acute phase response of tissues resulting in improved uterine health, whereas feeding the n-3 fish oil supplement reduced embryo loss after the first AI, and improved pregnancy rate after the second AI. The beneficial effect of feeding calcium salts of fish oil was augmented when calcium salts enriched in linoleic acid were fed previously during the transition period. The authors "proposed a strategy of FA supplementation comprised of feeding n-6 FA during the transition period, followed by EPA and DHA in the breeding period to maximize dairy cow production and reproduction".

Modifying Milk Fat Composition

In recent years there has been considerable interest to modify milk fat composition, to decrease the proportions of saturated fats and increase the more desirable or "healthy" fatty acids; of special interest among the latter being linoleic, linolenic and rumenic (CLA) acids. Although I am included among the guilty in this aspect, fortunately this craze has begun to wane, principally because investigators have run out of new ways to increase "CLA" in milk and more importantly, research in the last half dozen years showing that saturated animal fats are not the heart killers as portrayed by the medical community and the press for the past 50 years.

Bovine milk fat composition is highly plastic; modifying composition is accomplished by changing the fatty acid composition of the diet (Palmquist et al., 1993). It is not intended to give a detailed review here. For a sophisticated analysis of feeding various fats on milk fat composition, see Glasser et al., (2008). Feeding free fats or oilseeds that are biohydrogenated in the rumen increase the proportion of 18 carbon fatty acids in the milk, principally stearic and oleic acids, and decrease proportions of fatty acids with 6 to 16 carbons. Feeding supplements high in palmitic acid increase this and decrease mainly the C-18 fatty acids, depending upon amounts fed and level of production. Increasing oleic or linoleic acid, or both, lowers melting point of milk fat, whereas increasing palmitic acid increases melting point. Feeding calcium salts of unsaturated fatty acids may increase unsaturation of milk fat, feeding whole roasted soybeans increases oleic acid as well as both linoleic and linolenic acids (Morales et al., 2000a,b; Timmons et al., 2001). Fearon et al., (2004) fed oilseeds to fresh cows on spring grass in Ireland to produce a commercially successful soft butter. Feeding fish oil with unsaturated vegetable oil increases CLA to as great as 8% of fatty acids, but also increases undesirable trans fatty acids (Palmquist and Griinari, 2006).

CHARACTERISTICS OF RUMEN PROTECTED (BYPASS) AND RUMEN-INERT FATS

Rumen Protected Fat

The technical meaning of "rumen bypass" fat is one that passes directly to the omasum without entering the rumen. A "protected" fat may enter the rumen, but not interact with rumen metabolism. The only bypass fat is milk fat suckled by a newborn ruminant; among protected fats, I am aware only of the classical product developed in Australia nearly 50 years ago (Cook et al., 1970). That is a product in which fat is spray-dried or mixed with a soluble protein, which is then treated with formaldehyde to provide fat encapsulated in a protein matrix that is insoluble in the rumen, but solubilized in the abomasum at low pH. This process requires a careful balance between adequate formaldehyde for the product to not be degraded in the rumen, yet susceptible to acid. The result is an expensive product with varying quality. For a long time its commercial use was limited because of restrictions on use of formaldehyde in feedstuffs. It is my understanding that this restriction is no longer in effect. The main purpose in developing this product was to achieve a high level of unsaturated fatty acids in milk fat for softer butter (Wood et al., 1975), or as food for persons with hyperlipidemia (Brown et al., 1976). Although these ideas worked in research environments, they were not practical commercially, and are not in use today.

Rumen-Inert Fats

Rumen-inert fats may or may not be reactive in the rumen, but have the property to not inhibit microbial activity. Three types of commercial fat supplements fit this category: hydrogenated tallow, hydrogenated fatty acids, and calcium salts (soaps) of fatty acids. Hydrogenated fats have melting points above body temperature, and exist as solids in the rumen; further, hydrogenated tallow is poorly split by lipase activity, either in the rumen or intestine. Thus it is poorly absorbed (Table 2-3, NRC, 2001) and will not be discussed further. Fatty acids are adsorbed to surfaces of feed particles, and if unsaturated, are biohydrogenated by bacteria. Calcium soaps, as any salt, are subject to dissociation in the rumen; however the reaction is far to the right (pKdiss ~4.7; Sukhija and Palmquist, 1990), so >90% of dietary soap fatty acids and >60-75% of total fatty acids in the rumen exist in the insoluble soap form (Palmquist et al., 1986). That calcium soaps are ~50% biohydrogenated in the rumen (Wu et al., 1991) is evidence that dissociation has occurred, however, dissociated fatty acids do not achieve concentrations great enough to become inhibitory (Fig. 2).

THE FUTURE FOR RUMEN-INERT FATS IN DAIRY PRODUCTION

Finally, we come to the question of whether there is a viable future for rumen inert fats. At today´s level and potential for milk production of cows, it is imperative that fat be included to meet energy needs of the cow while maintaining optimal rumen function. Further, fat increases flexibility in formulation of dairy rations. As high energy sources, those rumen inert fats discussed above provide what is needed.

Rumen inert fat sources also provide other opportunities; for example, an inert source not mentioned above, palmitic acid alone, seems to have unique ability to increase milk fat percentage (Piantoni et al., 2013), which could be explained as a preference for palmitic acid to initiate esterification of glycerol and promote synthesis of short chain fatty acids in milk fat synthesis (Hansen and Knudsen, 1987a,b). Calcium soaps of unsaturated fatty acids provide opportunity to increase delivery of unsaturated fatty acids for improved reproduction, or possibly to modify milk fat composition. In my opinion, the latter provides little potential, whereas the former has a great future.

INERT FAT MARKETS

This last topic is one on which I have no expertise, but will offer some comment. Basically, use of manufactured rumen-inert fats for milk production is a mature market that will grow as milk production

increases, and especially as yield per cow increases. Some niche markets, mentioned above, could be use of palmitic acid to specifically increase milk fat production, and using calcium soaps of unsaturated fatty acids to manage the reproductive process of lactating cows could have great potential. Though I have no market data, from number of research papers published using different rumen-inert fats, calcium soaps may have the greatest market share. Estimates of the world markets for calcium soaps in the past 1–2 years are in the range of 350,000–400,000 tons annually. Using US/UK prices, the world market for calcium soaps approaches ½ billion dollars annually. From the number of companies offering various qualities of calcium soaps, it seems to me that the market is competitive.

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Update on Dietary Fat in Humans

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SUMMARY

- The links between dietary fat and specifically animal fat on human disease are primarily based upon *relative* risks, not *absolute* risks
    - On an absolute risk basis, the increased odds of developing a disease because of dietary fat are incredibly small.
- There are a variety of animal rights organizations that promote the supposed link between animal fat and human disease
- There is an increasing number of research trials that do not support the hypothesis that animal derived food products are unhealthy
- There is not a scientific consensus regarding dietary fat and human disease

INTRODUCTION

There has been and continues to be an unexplainable desire by dieticians and other "health" professionals to associate or link dietary components, particularly animal food products, with human disease (coronary heart disease, cancer, etc.). However, that perceived link is becoming increasingly ambiguous and is especially true with regards to ruminant derived products. In fact, not only is the link between dietary beef/dairy and disease suspect, there are a variety of micro-components in these high quality foods that are actually potent disease-fighting molecules.

Relative Risks vs. Absolute Risks

There are a variety of issues that makes conducting controlled (meaning there is an intervention and a control group) human experiments difficult (especially nutrition trials) and this is particularly arduous when the end measurement may be a life threatening disease. The first is the number of subjects necessary to gain statistical confidence, as disease incidence is often so low that it requires thousands of people (and a long period of intervention) to be enrolled in the experiment. The second is compliance, as humans are notorious for not following experimental dietary guidelines. The third is moral/ethical as humans certainly can not (or at least shouldn't) be administered a harmful molecule (i.e. a carcinogen) and then placed on a dietary regimen to see which treatment prevented/protected against the disease (as is normally done in animal models). Consequently, human nutrition trials rely heavily on "question" based data. An example question may be similar to the following; how many servings of a foodstuff (i.e. brussel sprouts, hot dogs….) do you eat on a weekly basis and have you been diagnosed with a specific disease during a period of time (i.e. 5 years). The respondents are then usually stratified (i.e. quintiles, etc.) into the quantity of the consumed food(s) of interest.

The difference in disease incidence between groups/quintiles can then either be presented as a relative difference or absolute difference. If the disease frequency in one Group A is 1/100 and the other is 2/100 then the absolute difference is 1% but the relative difference is 50% (Table 1). The 50% relative difference is an impressive number and headline grabber (especially compared to 1%) but it can be misleading (oftentimes intentionally) to readers not familiar with the data set. For example, alternatively (and probably a more rational and fair method of reporting the data) it could be presented as 99% of Group A and 98% of Group B people did not develop the disease. Would you alter your lifestyle, change your diet, take prescription medication, etc. to improve your chances of not getting a disease by 1%?

The studies linking dietary fat with human disease use statistics often times based on relative risks. When these "links" are evaluated on an absolute basis, it is clear the associations are incredibly weak at best (Taubes, 2001). In addition to the relationship in some studies being extremely low, some experiments are now indicating no risk of dietary animal fats (see below).

Table 1. Comparison of Data Analysis Methods: Relative vs. Absolute Risks

Disease Risk		Absolute Difference	Relative Difference
Group A	Group B	A - B	B/A %
20 % (2/10)	10 % (1/10)	10 %	50 %
2 % (2/100)	1 % (1/1000)	1 %	50 %
0.2% (2/1000)	0.1 % (1/1000)	0.1 %	50 %

http://www.acponline.org/journals/ecp/janfeb00/primer.htm

History of Nutritional Guidelines

The hypothesis that dietary fat is somehow deleterious to humans is over 50 years old. Ruminant lipid tends to be more saturated than other animal fats and this is especially true when compared to some common vegetable oils. The saturated fat content is the lightening rod for nutritionists and others as it has historically been the component identified as connecting diet and disease (Keys and Grande, 1957). Despite lacking a traditional scientific relationship (for an excellent description on the history of the dietary fat link with health, see review by Taubes, 2001) and regardless of recent reports contradicting the dogma, the 2000 Dietary Guidelines for Americans is as follows: "choose a diet that is low in saturated fat and cholesterol and moderate in total fat". The American Heart Association suggests to "choose foods like vegetables, fruits, whole-grain products and fat-free or low-fat dairy products most often" and the American Cancer Society indicates that "limiting saturated fat may be particularly important to reduce risk for both cancer and heart disease. Choose lean meats and low-fat dairy products, and substitute vegetables oils (like canola and olive) for butter or lard".

THE WOMENS HEALTH INITIATIVE (WHI) DIETARY MODIFICATION TRIAL

Until 2006, the reports linking diet, and specifically animal products, with cancer and other health disorders were primarily from epidemiological trials (Rose et al., 1986; WHO, 2003). Many of our national dietary recommendations are based on these international epidemiological trials. However, a large number of comparison trials recently published (within the last decade) do not support the hypothesis that dietary fat, specifically animal fat, increases the risk for cancer (Table 2) and it is perplexing as to why these reports are ignored by the American healthcare community. Nevertheless, comparison trials are limited by a number of scientific variables and results obtained should be used as initial suggestions for further randomized controlled investigations.

Table 2. Recent reports on the effects of total dietary and saturated fat on the incidence and relative risk of differing types of cancers.

Observ.	Cancer	Total Fat Risk	Saturated Fat Risk	Reference
4,980	Breast	↔	↔	Hunter et al., 1996
Cohort	Colorectal	↔	↔	Howe et al., 1997
Cohort	Breast	↔	↔	Lee & Lin, 2000
Cohort	Breast	↔	↔	Zock, 2001
Cohort	Colorectal	↔	↔	Zock, 2001
Cohort	Prostate	↔	↔	Zock, 2001
3,482	Breast	NR	↓	Shin et al., 2002
Cohort	Colorectal	NR	↓	Cho et al., 2004
910	Breast	NR	↓	Wirfalt et al., 2005
48,835	Breast	↔	↔	Prentice et al., 2006
48,835	Colorectal	↔	↔	Beresford et al., 2006
1,123	Skin	↓	NR	Granger et al., 2006

↔: no relationship
↓: Decreased risk
NR: Not reported
Cohort: a review of multiple trials

The WHI trial was designed in the early 1990's as a randomized controlled study with the goal of definitively testing the effects of dietary fat and its specific components on a variety of human diseases. The trial included more than 160,000 women (50-79 years old) from 40 different centers across the country, lasted for approximately 8 years and cost more than $700 million dollars. The women were either assigned to a low fat diet (while simultaneously increasing vegetable and fruit intake) or advised to stay on their usual eating pattern. Women on the low-fat diet had saturated fat intakes that represented about 7% of their total energy intake. Results from the largest and most comprehensive study on dietary fat in American history indicate that there is NO relationship between either total dietary fat or saturated fat on the incidence/risk of colorectal (Beresford et al., 2006) or breast (Prentice et al., 2006) cancer or on cardiovascular disease (Howard et al., 2006).

It is unclear why there are so many inconsistencies in the epidemiological literature with regards to dietary fat, specifically fat from animal products, on human health. The fact that there are such large inconsistencies makes it especially confusing as to how the dietary fat dogma became entrenched in the medical community. Regardless, the recent WHI controlled experiment should (in addition to the latest reports in Table 1) assist in creating new and more accurate nutritional guidelines and provide strong evidence as to why milk and other ruminant food products should remain an important part of a balanced healthy diet.

It is important to note that many organizations appearing to be concerned with public health (Table 3) may actually front for animal rights groups (i.e. People for the Ethical Treatment of Animals: PETA; Animal Liberation Front: ALF). They have unsuccessfully persuaded the general American public that consuming animal products is immoral and unethical, but convincing consumers that the products are unhealthy is an alternative means to an end (elimination of animal agriculture). An example is the Physicians Committee for Responsible Medicine (incidentally, less than 5% of its members are physicians; Newsweek, 2004), which advocates that a vegetarian diet reduces the risk of cancer and other health disorders as stated on their website: "vegetarian foods may help prevent cancer and even improve survival rates". These groups have done an excellent job of convincing the public and media that they are legitimate scientists and actual health care professionals with a genuine concern for the public health.

Table 3. "Health organizations" that recommend decreasing animal food product consumption

Organization	Website
Center for Food Safety	www.centerforfoodsafety.org
Center for Science in the Public Interest	www.cspinet.org
Physicians Committee for Responsible Medicine	www.pcrm.org

ANTICARCINOGENS IN RUMINANT FOOD PRODUCTS

Numerous studies have been conducted with various human cancer cell lines and animal models showing that milk components can prevent the development and progression of cancer (see review: Gill and Cross, 2000). Many of these components are in the milk fat fraction and include butyric and vaccenic acids, ether lipids, sphingomyelin, Vitamin A and carotene (Parodi 1997). An additional molecule receiving considerable attention and the one most extensively studied is conjugated linoleic acid (CLA). For a detailed description on CLA ability to prevent different types of cancer, see recent reviews (Belury, 2002; Ip et al., 2003)

CLA describes positional and geometric isomers of linoleic acid, with the double bonds being separated by a single methylene group. CLA are synthesized in the rumen through biohydrogenation of polyunsaturated fatty acids and therefore are found naturally in dairy products and ruminant meat (Bauman et al., 2001). The *cis*-9, *trans*-11 isomer is the most abundant CLA isomer found in ruminant products, though both *cis*-9, *trans*-11 and *trans*-10, *cis*-12 have shown anticarcinogenic properties (Ip et al., 2003).

Although there is a wealth of evidence demonstrating that synthetic, purified CLA isomers have anti-cancer properties, recent attention has turned to CLA effects when presented as it would be in a normal diet (at smaller concentrations and in combination with many other fatty acids). In a recent study, mice were fed CLA (*cis*-9, *trans* -11/*trans*-10, *cis*-12 mixture) in combination with either a vegetable oil blend, corn oil, or beef tallow. Data indicate that CLA was more effective at decreasing tumors when beef tallow was added to the diet (Hubbard et al., 2006). Additionally, fatty acids extracted from beef (<1% CLA content) had a greater anti-proliferative effect on cancer cells than a synthetically enriched CLA diet (De La Torre et al., 2006). Collectively, these trials suggest CLA found naturally in ruminant-derived products may potentially be significant contributors to a healthy and cancer preventive diet.

Increasing the CLA content in ruminant products

CLA is an intermediate in rumen biohydogenation of linoleic acid (C18:2; Bauman et al., 2001), but it primarily derived via desaturation of vaccenic acid (*trans*-11 C18:1, also a product of rumen polyunsaturated fatty acid biohydrogenation) by the Δ^9-desaturase enzyme (Corl et al., 2001; Kay et al., 2004). Vaccenic acid is also an intermediate of linolenic acid (C18:3) biohydrogenation (Bauman et al., 2001) so including both fatty acids in the diet of ruminant animals has the potential to increase the rumen output of *trans*-11 C18:1 and thus enhance the content of *cis*-9, *trans*-11 CLA in food products.

The milk fat CLA content from TMR-fed cows can markedly be increased (i.e. $\geq$ 5-7 fold) by adding a variety of plant oils (i.e. sunflower, linseed etc.) to dairy rations. Altering the oils with TMR-fed cows can increase the CLA content so that it is equal to or greater than that found in pasture-fed cows (which typically have an enhanced CLA content, Kelly et al., 1998). For a detailed description on successful methods to enhance the CLA content in dairy products see a recent review (Lock and Bauman, 2004).

DAIRY CALCIUM AND WEIGHT LOSS

Milk is a rich source of a number of vitamins and minerals (potassium, chloride, sodium, calcium etc.) that are required in the human diet such as fat-soluble vitamins (A, D, E, and K), as well as the B vitamin

family, specifically thiamin, riboflavin, B_6, and B_{12}. Recently, calcium intake, particularly from dairy sources, has been implicated in decreased incidence of obesity within the human population. Dietary calcium is crucial to the regulation of energy metabolism, in that it has been found to attenuate adipocyte lipid accretion during over consumption of energy-dense diets, as well as to increase lipolysis and preserve thermogenesis during caloric restriction, leading to accelerated weight loss (Zemel, 2003). It has been demonstrated that calcium supplementation, in rodent and human models, decreases visceral adiposity, a precursor to the metabolic syndrome (Zemel et al., 2004; Azadbakht et al., 2005; Liu et al., 2005). The proposed mechanism of action for the role of calcium in decreasing adiposity is that supplementation of calcium results in a reduced concentration of intracellular calcium, via suppression of 1,25-(OH_2)-D, which leads to a coordinated deactivation of fatty acid synthetase (Sun and Zemel, 2004) and an increase in lipolysis (Shi et al., 2001; Zemel, 2001). In addition, it might also increase uncoupling proteins and thus increase metabolic heat production (Shi et al., 2001) and this might be the mechanism by which dairy products help with weight loss even though these people are not necessarily on a lower calorie diet.

A number of studies have been conducted utilizing calcium to modulate obesity ranging from epidemiological and observational studies to those investigating the mechanism of action utilizing a transgenic obese mouse model. It has been demonstrated that a dairy source of calcium, rather than a synthetic supplemental source such as calcium carbonate, has greater impacts on weight loss (Zemel et al., 2000, 2004). In a study conducted by Zemel and coworkers (2004), it was demonstrated that calcium supplementation in obese adults, particularly in the form of dairy products, significantly increased weight loss, decreased body fat percentage and reduced waist circumference. Furthermore, individuals consuming high calcium diets in the form of dairy products had a significant reduction (44%) in circulating insulin levels. In a study conducted by Liu and co-workers, (2005), it was determined that consuming dairy products in middle-aged and older women was associated with a decreased incidence of metabolic syndrome. Women consuming a high calcium diet (>1,500 mg/day) exhibited decreased waist circumference, BMI, hypertriglyceridemia, high blood pressure, and incidence of type 2 diabetes and increased HDL cholesterol.

Recent evidence demonstrates that calcium has an anti-obesity effect, particularly when it comes in the form of dairy products. Utilizing yogurt, or non-fat dry milk in studies, regardless of the subject (rodent or human), increased weight loss and decrease fat percentage to a greater extent than calcium from a synthetic source such as calcium carbonate. Milk is a rich source of many bioactive compounds which either act independently or synergistically with calcium to accelerate lipolysis and/or effect nutrient partitioning between adipose tissue and skeletal muscle. Therefore, supplementation of calcium, in the form of low-fat dairy products, attributes to increased weight loss.

SUMMARY

The link between dietary fat and specifically fat derived from ruminant animals, with human disease is incredibly small at best and probably does not exist. Unfortunately, the hypothesis that animal food products are “unhealthy” has become dogma in popular culture (driven in part by organizations with ulterior and covert motives) and even people with little or no biological knowledge now affiliate ruminant food products with “heart attacks” and “cancer”. In stark contrast to the “doom and gloom” message we have consistently heard from the medical community and dieticians for the past four decades, there are a variety of micro-components in dairy and beef products that are strongly associated with prevention and treatment of disease (cancer, obesity, etc.). A coordinated and concerted effort by agricultural and biological scientists AND the animal agriculture industry is necessary to re-educate consumers about biology and nutrition.

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Veterinary Feed Directive: History, Responsibilities and Update

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SUMMARY

Presentation will focus on the following:

- History of the Veterinary Feed Directive (VFD)
  - Key terms
  - Key documents
- Current rules for implementing the Veterinary Feed Directive
  - Veterinarian
  - Producer
  - Feed mill and distributors
- Proposed Changes to the current rules for implementation of the Veterinary Feed Directive

INTRODUCTION

The Veterinary Feed Directive (VFD) was passed in the Animal Drug Availability Act of 1996 and the Food and Drug Administration (FDA) proposed regulations relating to the distribution and use of VFD and animal feeds containing VFD drugs in July 1999. Since then a number of guidance documents for industry (#209 & #213) and the VFD rules were published with the 21 CFR 558 providing details for implementing the VFD process for use of antibiotics in animals. In December of 2013, the FDA announced the implementation of its plan to phase out the use of medically important antimicrobials in food animals for production purposes and when these classes are used for therapy their use will be supervised by a veterinarian. We can all agree it's in everyone's best interest to have access to effective antibiotics for people and animals. It is critical for public health and vital for livestock production and well-being. It is important for those of us in agriculture to do our part in protecting antibiotic effectiveness and help maintain confidence in food safety. These VFD progressions are part of the solution. This paper will focus on summarizing the different documents and VFD rules as we understand them today.

HISTORY

In 2012, the U.S. Food and Drug Administration (FDA) put forth plans to change the way feed and water-based antimicrobials are used in livestock production. Three documents provide the details: Guidance for industry No. 209, Guidance for Industry No. 213 and Veterinary Feed Directive (VFD) 21 CFR 558. The first one, (# 209) is the "what" component. It establishes the key principles: The use of medically important antimicrobial drugs in food-producing animals should be limited to using it only when necessary for assuring animal health and includes veterinary oversight.

The second report (# 213) is the how component. It provides a road map for implementing those two principles by addressing issues around product labeling for the sponsors…

The third report (21 CFR 558) aims to modernize the VFD process. It is an effort to streamline procedures while providing greater oversight. While VFDs have been used for over ten years for certain products, with this great increase in number of antibiotics moving to VFD status this modernization will be critical for practicality and to lessen the possibility that needed antibiotics be denied due to the paperwork mechanics.

FDA is taking these steps to protect public health, on that front it is the right policy for the right reasons. The FDA calls antimicrobial resistance "a mounting public health problem of global significance". The FDA fears that if certain antimicrobials are overused in animals they'll become less effective in humans.

With the release of these reports, the agency said it was pursuing a "voluntary approach" for compliance. While the process to change labels is voluntary once the changes are made compliance by farmers, veterinarians and feed manufacturers would not be voluntary but mandated by law. The good signal is that all sponsors have given written notice to the FDA that they will comply. The FDA plans to evaluate progress and expects completion three years after the final publication and "consider further action as warranted." Guidance for industry #213 was finalized in Dec 2013 so the clock is ticking.

From an industry perspective this might not seem ideal. However, it's preferable to many alternatives such as elimination of medications or Congressional oversight. There would be challenges with those as well.

These steps go a long way toward protecting long term access to antibiotics. The voluntary approach gives everyone time to understand the policies, figure out how to comply with them and determine the most efficient process for transitions.

In modern livestock production antibiotics are used in four ways.

Therapy:

- Treat animals diagnosed with an illness
- Control the spread of an illness in a herd
- Prevent illness in healthy animals

Production:

- Enhance growth or improve feed efficiency by increased utilization of nutrients

The FDA says that "medically important" antibiotics (drugs considered important for therapeutic used in humans) would still be available for those first three therapy-related uses under the supervision of the veterinarian. But these products could not be used for enhancing growth or improving feed efficiency.

Basically there are three classes of antibiotics: **Human-only antibiotics** are not approved for use in animals, creating a reserve of unique antibiotics for human health needs; Because animals are susceptible to different diseases and have different needs than humans, **animal-only antibiotics** have been developed to treat specific health requirements of animals and are not used in human medicine **Shared-class antibiotics** are approved for animals and humans. These shared-class of antibiotics are the ones impacted by the recent guidance.

Some examples of drugs deemed "important for human medicine" and used in both animals and humans that would require a VFD are: Penicillins, Cephalosporins, Quinolones, Fluorquinolones, Tetracyclines, Macrolides, Sulfas, Glycopeptides and others.

Examples of drugs NOT requiring a VFD are: Ionophores, Polypeptides, Carbodox, Bambermycins and Pleuromutilin.

To learn more about antimicrobial resistance and which products are affected, see www.FDA.Gov/animalveterinary/safetyhealth/antimicrobialresistance/default.htm

IMPLEMENTATION

Three main groups responsible for the VFD process and record keeping are the veterinarian, producer and the feedmill or feed distributor. Veterinary oversight in the use of medically important drugs is a key for the VFD process to work.

For a VFD currently a veterinarian must:

1. Have a valid veterinary license in the state where the animals being fed the medicated feed are located.
2. Have a valid Veterinarian-Client-Patient Relationship (VCPR) with the flock or herd being fed the VFD feed. The requirements of a VCPR may vary from state to state however, the American Veterinary Medical Association Principles of Veterinary Medical Ethics state it is unethical for a veterinarian to write a prescription or dispense a prescription drug outside a VCPR. Some of the principles include: Responsible for sufficient knowledge of animals to make a preliminary diagnosis and personally acquainted with the care of the animals and timely visits to where the animals are managed, providing oversight of treatment and compliance of treatment instructions, readily available for follow up examination and maintain records on the animals.
3. Completely and accurately fill out and sign the VFD orders. May electronically sign if using an electronic VFD (eVFD) process. A eVFD must be sent using a computer system that is compliant with Title 21, Part 11 of the Code of Federal Regulations (21 CFR 11). (Note: Telephone orders are not allowed)
4. Provide the feed mill or distributor with the original VFD order. Can fax or scan and email a signed order to feed mill but then must provide the original signed VFD order within 5 working days. An eVFD has an authorized electronic signature and therefore no follow up paper copy is required.
5. Provide the producer with a copy of the VFD or eVFD order
6. Keep a copy of the VFD order for a minimum of two years
7. Have the VFD orders available for an FDA inspection

Many of the VFD drug sponsors (although not required) provide VFD order forms for their products. A veterinarian may develop their own form. To be a valid VFD form, currently it must contain the following information:

1. Veterinarian name, address, telephone and fax numbers
2. Veterinary license and state where issued
3. Clients name, address and telephone number
4. Animal species and number of animals being fed the medicated feed
5. A description of animals and identification number
6. Location of the animals
7. Date the VFD order is issued and date of treatment
8. Name of the animal drug
9. Drugs approved indication for use
10. Level of drug in the feed and amount of feed need to treat the animals
11. Feeding instructions and withdrawal times (if applicable)
12. Any special instructions and cautions statements needed for the drug
13. The VFD order expiration date
14. Number of refills (reorders) allowed under the drugs approved regulation
15. Must have the following verbatim statement “Extra-label use (i.e., use of this VFD feed in a manner other than as provided for in the VFD drug approval) is strictly prohibited"
16. Any other information the VFD drugs approval regulation requires to be on the form.

The veterinarian must complete the VFD in writing and sign it. Any VFD orders that are incomplete or unsigned are considered an invalid order and the feed mill or distributor will not manufacture the medicated feed. The feed mill or distributor are not required to contact the veterinarian for incomplete or unsigned VFD orders for correction. The veterinarian is responsible for the completeness and accuracy of the VFD order and needs to make sure the original paper VFD order is given to the feed mill or distributor within 5 working days.

GlobalVetLink, a health data management company has created an electronic Veterinary Feed Directive system (eVFD) for veterinarians that allows for completion of the necessary paperwork with a number of benefits for the veterinarian, producer and feed mill. These benefits include: ease of filling out the paperwork, avoiding miscalculation of feed required, automatically sending copies to producer and feed mill, easy renewal when necessary and maintaining a secure documentation of the VFD. All these benefits combine to speed the process and help the veterinarian's commitment to providing excellent care for animals to produce a safe supply of protein.

Producer

The producers have current responsibilities in the VFD process as well including:

1. Contact a veterinarian to diagnosis if animals need treatment and with what VFD drug would be used. If a VFD drug is needed, the veterinarian must complete the VFD order to give to the producer or directly to the feed mill or distributor.
2. Follow the veterinarian's recommendations and administrating the VFD medicated feeds to the identified animals according to the directions of the VFD order. Extra-label use (i.e., use of VFD feed for unapproved indications or unapproved doses) is strictly prohibited.
3. Keep a copy of the VFD order(s) for at least two years and have order(s) for FDA inspectors when requested.

Feed Mills and Distributors

Feed mills and distributors responsibilities currently include:

1. Maintain a valid FDA feed mill license application and renewing it electronically every year. The mill will manufacture the feed according to the dosage of the drug and amount of feed required to treat the animals.
2. Keep all original VFD orders for two years from date of issue and provide all orders on file upon an FDA inspection.
3. Submit to FDA a one-time distribution notification letter to the FDA indicating intention to distribute VFD drug-containing medicated feed.
4. Keep acknowledgement letters from all purchasers of VFD feeds who are not the end-users of the VFD feeds. These acknowledgement letters must state that purchasers will only sell VFD feeds to producers with a valid VFD orders or to other distributors whom have acknowledgement letters.
5. Ensure all labels and advertisements for VFD feeds distributed by the feed mill or distributor contain the following statement verbatim "Caution: Federal law limits this drug to use under the professional supervision of a licensed veterinarian. Animal feed bearing or containing this veterinary feed directive drug shall be fed to animals only by or upon a lawful veterinary feed directive issued by a licensed veterinarian in the course of the veterinarian's professional practice."
6. Keep current approved Type B and/or Type C medicated feed labels for each Type B and/or Type C medicated feed mill will be manufacturing before receiving the VFD drug containing Type A medicated feed from drug manufacturer.
7. Notify the FDA within 30 days when making changes to your business name or address

WHAT CHANGES MAY OCCUR BEFORE 2017 IMPLEMENTATION

It has been over a decade since FDA began implementing the final rule relating to VFD drugs and there are very few currently approved VFD drugs. The process continues to be refined so it isn't too burdensome for implementation. The proposed changes were published in Federal register of March 29 2010 (75 FR 15387) for comments until March 12, 2014. These comments are being considered and a final rule is predicted to be finalized in the first half of 2015. Some of the FDA proposals include the following:

1. Make the VFD rules easier to understand by reducing the number of subsections from 6 to 3. This will be accomplished by providing general rules that are common to all three stakeholders (veterinarian, producer and feed mills). A section on rules specific for veterinarian including completeness and accuracy of VFD forms. The third section for the feed mill or distributor to assure the medicated feed is manufactured and labeled in accordance with the VFD approved conditions of use.
2. Increased flexibility for veterinarians issuing the VFD by removing the explicit VCPR and replace it with a requirement those veterinarians to be in compliance with all applicable veterinary licensing and practice requirements. This removes the "one-size-fits-all" Federal standard deferring to the individual state and the veterinary profession for specific criteria acceptable veterinary professional conduct. This is especially important in remote geographical areas and the veterinarians work with other health professionals such as biologists or pathologists.
3. Veterinarians may only issue a VFD for use of VFD drugs in animals under their supervision or oversight
4. Current rules do not allow an unlicensed feed mill to manufacture a VFD medicated feed from a VFD Type A medicated feed product. Under the current VFD regulations, all medicated feed distributors, licensed or unlicensed, are able to manufacture and sell medicated feeds containing VFD drugs. The only difference is that licensed facilities are able to start the manufacturing process with a VFD Type A medicated article and unlicensed facilities must start with a VFD Type B [(3)] or Type C [(4)] medicated feed. In other words, unlicensed feed mills are not allowed access to *any* VFD Type A medicated articles under current regulations. FDA proposes to amend the VFD regulations to allow unlicensed feed mills to have continued access to the Type A medicated articles they currently use when these drugs change from OTC to VFD status.
5. Remove the requirement the veterinarian include the amount of medicated to be dispensed because of the difficulty in knowing exactly how much medicated feed a set of animals will need especially taking in consideration all different factors including environmental, variability in health status of the animals and respond to the VFD drugs. Overestimation of feed needed would result in wasted feed or underestimation of feed would result in a need for another VFD order.
6. Reduce record retention from 2 years to 1 year for the VFD feed orders. This will eliminate the discrepancy between VFD medicated feeds and other feed record retention requirements. Also allow all of the records to be maintained electronically.

Follow changes between now and final implementation of the Veterinary Feed Directive by contacting drug sponsors with a VFD drug of interest or seek information from FDA website at http://www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/JudiciousUseofAntimicrobials/default.htm

Some key documents found on this site include:

CVM GFI #209 - The Judicious Use of Medically Important Antimicrobial Drugs in Food-Producing Animals

CVM GFI #213 - New Animal Drugs and New Animal Drug Combination Products Administered in or on Medicated Feed or Drinking Water of Food-Producing Animals: Recommendations for Drug Sponsors for Voluntarily Aligning Product Use Conditions with GFI #209

Veterinary Feed Directive Proposed Rule published December 12, 2012

Moving forward antibiotic use in feed and water for food animals are not being eliminated but how and the process to use them will be altered. A system that insures and demonstrates responsible use, keeping aware of the changes and preparing for the change would be wise. Working together through education and more advanced technology and specific tools, these changes will be the right thing for the producer, veterinarian, consumer and while still providing access to antibiotics when needed for protecting animal well-being, food safety, and sustainability.

Fat Supplementation: Are All Fatty Acids Created Equal?

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SUMMARY

- An extensive metabolism of dietary unsaturated fatty acids in the rumen results in the lipid material leaving the rumen consisting primarily of free fatty acids that are highly saturated.
- Fatty acid supplements are commonly fed to increase dietary energy density and support milk production.
- In general fatty acid supplementation increases milk yield, milk fat yield, and the efficiency of milk production, however great variation has been reported in production performance for different fatty acid supplements, and indeed the same supplement across different diets and studies.
- Not all fatty acids are the same: know what fatty acids are in the supplement and what form they are in; interactions with other dietary components are key in determining response.
- Always consider potential effects of fatty acid supplements on feed intake, milk production, milk composition, body condition, and energy partitioning.
- Further work is required to characterize the sources of variation in response to fatty acid supplementation.

INTRODUCTION

In most Federal Milk Market Orders milk fat and protein content are the major contributors to the price that producers receive for milk. In an economic analysis assessing the value of milk components for the past ten years, a 5% increase in fat yield, protein yield, and milk yield increased net farm income by 13%, 15%, and 3%, respectively (St-Pierre, 2011 ADSA Discover Conference). The addition of supplemental fatty acids (FA) sources to diets is a common practice in dairy nutrition to increase dietary energy density and to support milk production. Understanding the effects of different types of FA supplements on production parameters has direct impact on dairy industry recommendations and the usefulness of FA supplementation strategies. The emphasis of the current paper is to provide a general overview of lipid metabolism in the dairy cow along with a discussion on the potential use of FA supplements in dairy cattle rations. Focus will include biological processes and quantitative changes during the metabolism of FA in the rumen and the effect this has on FA availability to the dairy cow. Information will be provided on the impact of FA supplementation on cow performance and the specific effects of supplements with different FA profiles on feed intake, milk production, milk composition, and energy partitioning.

LIPID METABOLISM IN THE RUMEN AND MAMMARY GLAND

As well as being derived from specific supplements, FA in the dairy cow's diet are also present in forages and concentrates. The FA composition of some typical feedstuffs is shown in Table 1. Each feed/fat source is composed of a different mix of individual FA. Generally, most cereal grains and seeds contain a high concentration of linoleic acid (18:2 n-6), whereas linolenic acid (18:3 n-3) is typically the predominant FA in forage sources. For example, corn, cottonseed, safflower, sunflower, and soybean oils are high in linoleic acid, whereas linseed is high in linolenic acid. Fish oil contains eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), two very long chain n-3 FA that are gaining interest due to their potential impact on animal health and reproduction. Unsaturated FA are toxic to many rumen bacteria, thus an extensive metabolism of dietary lipids occurs in the rumen that has a major impact on the profile of FA available for absorption and tissue utilization (Palmquist et al., 2005). The two major processes that occur are hydrolysis of ester linkages in lipids found in feedstuffs and the biohydrogenation (BH) of

unsaturated FA (Figure 1). BH of unsaturated FA results in the conversion of unsaturated FA to saturated FA, mainly stearic acid (18:0), through a series of BH intermediates (conjugated 18:2 and *trans* 18:1 FA). The major substrates are linoleic and linolenic acids and the rate of rumen BH is in the range of 70-95% and 85-100%, respectively (Jenkins et al., 2008); thus stearic acid is the predominant FA available for absorption by the dairy cow under typical feeding situations (Bauman and Lock, 2006). A series of recent in vitro studies concluded that BH occurs to enable rumen bacteria to survive the bacteriostatic effects of unsaturated FA, and that the toxicity of unsaturated FA is probably mediated via metabolic effects rather than disruption of membrane integrity. Furthermore, it appears that the degree of toxicity of different unsaturated FA varies for individual ruminal bacteria species; all the main species that comprise the ruminal cellulolytic bacteria appear vulnerable to inhibition by unsaturated FA (Maia et al., 2007, 2010).

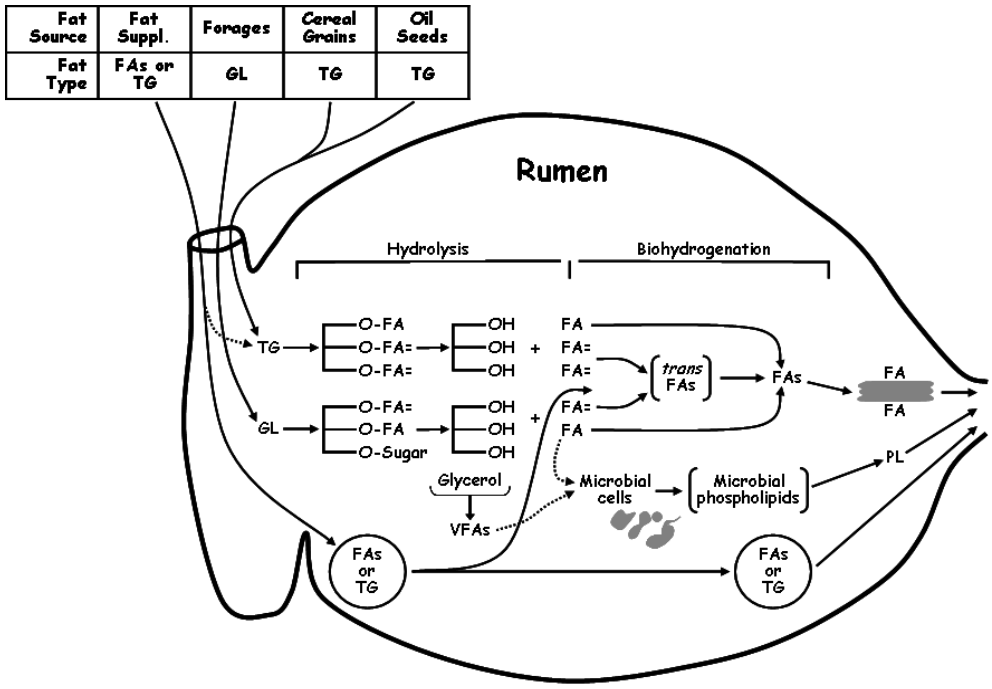
Table 1. Representative fatty acid composition of common feedstuffs (Data from CPM Feed Library).

Feed Name	Fatty Acid (g/100g fatty acids)					
	C14:0 Myristic	C16:0 Palmitic	C18:0 Stearic	C18:1 Oleic	C18:2 Linoleic	C18:3 Linolenic
Corn Silage	0.46	17.8	2.42	19.2	47.7	8.25
Alfalfa Silage	0.66	18.8	3.35	2.05	15.9	38.7
Grass Hay	0.43	16.4	1.33	2.53	23.4	49.9
Corn Grain	2.33	13.2	1.99	24.1	55.7	1.62
Soybean Oil	0.11	10.8	3.89	22.8	53.8	8.23
Corn Distillers	0.14	14.1	2.39	24.6	56.1	1.68
Cottonseed	0.69	23.9	2.33	15.2	56.5	0.19

Improvements in analytical techniques have revealed an impressive complexity in the pattern of FA that are produced during rumen BH and subsequently incorporated into milk fat. The established major pathways of BH describe the formation of *trans*-11 18:1 and *cis*-9, *trans*-11 CLA, but do not account for the FA intermediates arising from minor pathways of rumen BH (Palmquist et al., 2005). This is an area of increasing interest because of the recognition that some of these BH intermediates have specific and potent effects on ruminant metabolism and human health. For example, both *trans*-11 18:1 and *cis*-9, *trans*-11 18:2 present in milk fat have been shown to have anticarcinogenic and antiatherogenic properties in animal models of human health (Lock et al., 2009), while the role of *trans*-10, *cis*-12 18:2 as a regulator of milk fat synthesis is well established (Bauman et al., 2011).

FA supplements are often used as a means to increase the energy density of the diet and many of these are referred to as inert. In this case inertness simply means that the FA supplement has minimal affects on rumen fermentation. Although deemed inert at the level used, they can still be hydrolyzed, if a triglyceride, or biohydrogenated, if unsaturated (Figure 1). Often, calcium (Ca) salts of palm FA or canola are referred to as 'protected'. However, these are not protected from ruminal BH, but rather are considered to be ruminally inert with regard to their effects on the microbial population (Palmquist, 2006).

Figure 1. Lipid metabolism in the rumen. Also shown are the predominant fat types in common feedstuffs (TG = triglycerides, GL = glycolipids and FA = fatty acids).



Lipids in milk are primarily in the form of triglycerides (98%) with phospholipids and sterols accounting for 1.0 and 0.5 % of total lipids, respectively. Bovine milk is extremely complex and contains about 400 FA, a large proportion of which are derived from lipid metabolism in the rumen (Jensen, 2002). Milk FA are derived from 2 sources; <16 carbon FA from de novo synthesis in the mammary gland and >16 carbon FA originating from extraction from plasma. 16-carbon FA originate from either de novo or preformed sources. Substrates for de novo synthesis are derived from ruminal fiber digestion and dietary FA supply preformed FA for direct incorporation into milk fat (Palmquist, 2006). Microbial synthesis of branched and odd-chained number FA in the rumen and absorption of BH intermediates also contribute to the diversity of FA secreted in milk fat. Under typical conditions, about half of the FA in milk are synthesized de novo, 40 to 45 % originate from FA in the diet, and less than 10% are derived from mobilization of adipose tissue (Palmquist and Jenkins, 1980). However, nutrition can substantially alter the balance between mammary de novo FA synthesis and uptake of preformed FA.

USE OF SUPPLEMENTAL FAT: IMPACT OF DIFFERENT FATTY ACIDS

The addition of supplemental FA sources to diets is a common practice in dairy nutrition to increase dietary energy density and to support milk production. There is a wide range of FA supplements available for lactating dairy cattle. For example, Ca-salts of free FA and prilled saturated free FA are two common types of supplements used in the dairy industry and they differ in FA content and FA profile. Ca-salt supplements typically contain 80-85% FA and these typically provide approximately 50% are saturated and 50% unsaturated FA. By comparison prilled saturated free FA contain approximately 99% FA which are approximately 90% saturated, 10% unsaturated. A summary of the FA profile of some commonly used supplements is provided in Table 2. Although in general FA supplementation has been shown to increase milk yield, milk fat yield, and the efficiency of milk production, great variation has been reported in production performance for different FA types, and indeed the same supplement across different diets and studies. This is evident in a meta-analysis examining the effect of FA supplementation to diets of dairy

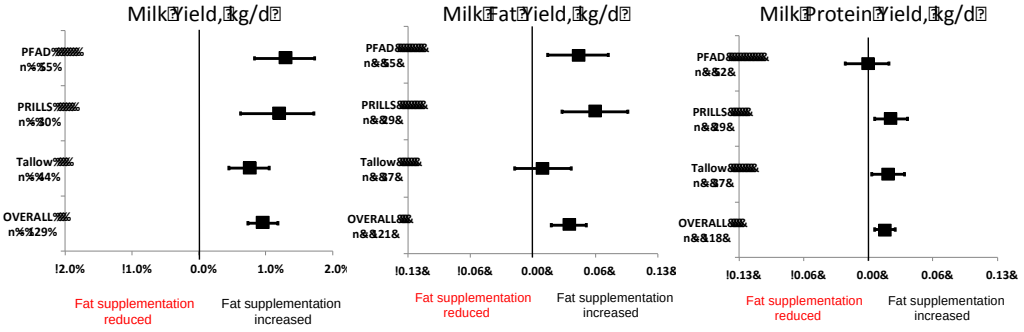
cows (Rabiee et al., 2012). In general milk production and milk fat % and yield increased, DMI and milk protein % decreased, and milk protein yield was not affected by FA supplementation. There was a wide range of responses (~5 standard deviations) for all variables, indicating varied and marked biological effect of the different FA supplements (Rabiee et al., 2012).

Table 2. Fatty acid composition of common fat supplements (Data from our laboratory).

Fatty Acid, g/100 g	Tallow	Ca-salt PFAD	Saturated free FA	C16:0-enriched
C14:0	3.0	2.0	2.7	1.6
C16:0	24.4	51.0	36.9	89.7
C18:0	17.9	4.0	45.8	1.0
C18:1	41.6	36.0	4.2	5.9
C18:2	1.1	7.0	0.4	1.3

Utilizing a larger data set than Rabiee et al. (2012), we recently performed a meta-analysis of production responses to commercially available FA supplements (Boerman and Lock, 2014a). Available data were collected from 133 peer-reviewed publications of which 88 met our selection criteria, comprising 159 treatment comparisons. Calcium-salts of palm FA distillate (PFAD; n=73), saturated prilled FA (PRILLS; n=37), and tallow (n=49) supplemented at ≤ 3% diet DM were compared to non- FA supplemented diets used as controls. Treatment comparisons were obtained from either randomized design (n=99) or crossover/Latin square design experiments (n=60). Preliminary results from the meta-analysis are shown in Figure 2. Overall, FA supplementation increased yield of milk and milk components and reduced DMI. However type of supplement influenced response with PRILLS not reducing DMI, tallow having no effect on milk fat yield, and PFAD having no effect on milk protein yield. It is important to note that the majority of the studies reported in Figure 2 simply compared a single commercial FA supplement with a non- FA supplemented control diet. This makes direct comparisons between different FA supplements difficult to interpret and importantly provide accurate answers to a commonly asked question (by farmers and nutritionists) as to which are the best FA supplements to use. There are limited reports in the published literature that have undertaken direct comparisons between different commercially available FA supplements.

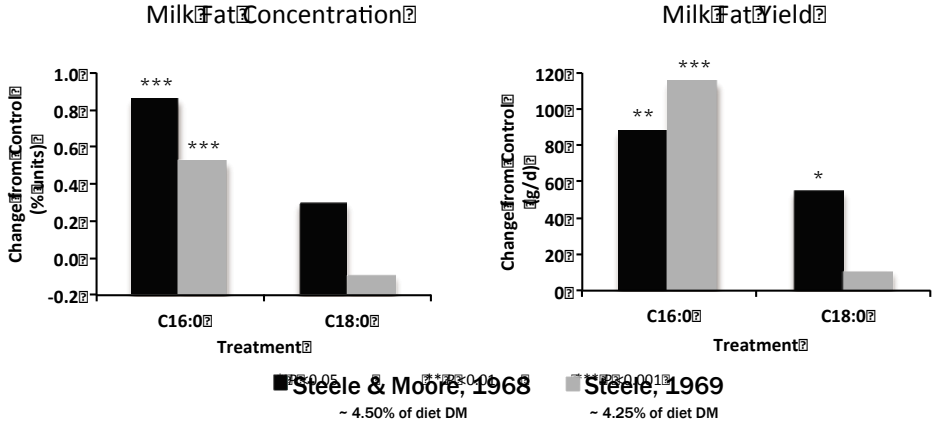
Figure 2. Effect of commercially available fatty acid supplements on yield of milk, milk fat, and milk protein (Boerman and Lock, 2014a). All data reported in peer-reviewed journals in which FA supplements were included at ≤ 3% diet DM compared to control with no added FA supplement. All studies had to have measurements of variance reported. **PFAD** – calcium salts of palm FA distillate (~ 50% 16:0, ~ 50% unsaturated 18-carbon FA); **PRILLS** – saturated FA prills (> 80% saturated FA [16:0 and/or 18:0]); **Tallow** – animal fat labeled as tallow (~ 50% 16:0 and 18:0, ~ 45% 18:1). Data analyzed using Comprehensive Meta-Analysis (CMA) version 2.0 (Biostat, Englewood, NJ), calculating difference between FA supplemented and control diets using a random effects model.



Total milk fat yield as well as fat percentage is often increased when saturated FA supplements are fed. Christensen et al. (1994) compared the effects of abomasal infusion of saturated long chain FA and unsaturated long chain FA (high oleic canola oil, soybean oil, and sunflower oil) and found that saturated FA infusion increased milk fat yield as compared to the unsaturated FA infusions. These findings are similar to those of Relling and Reynolds (2007) who reported an increase in milk fat percentage and yield as a result of feeding saturated FA compared to polyunsaturated (Ca-salts of soybean FA) and monounsaturated (Ca-salts of palm FA distillate) FA treatments; furthermore the saturated FA treatment increased milk fat compared to the non- FA supplemented control treatment.

These aforementioned results lead to important questions as to whether different saturated FA have different effects when fed to dairy cows. There is evidence from the 1960's that individual saturated FA are more or less effective at increasing milk fat yield. Steele and co-workers performed a series of studies using relatively pure sources of palmitic, oleic, and stearic acids and their findings suggest that palmitic acid supplementation induces a higher milk fat response (concentration and yield) as compared to oleic and stearic acids supplementation (Figure 3). More recent work from Enjalbert et al (1998) suggests that the uptake efficiency of the mammary gland is higher for palmitic acid than for oleic and stearic acids. We recently carried out a series of studies examining the effect of individual saturated FA on production and metabolic responses of lactating cows (Lock et al., 2013, Piantoni et al., 2013, Rico et al., 2014, Piantoni et al., 2015). These results indicated that palmitic acid supplementation has the potential to increase yields of milk and milk fat as well as the conversion of feed to milk, independent of production level when it was included in the diet for soyhulls or stearic acid. Piantoni et al. (2015) reported that stearic acid increased DMI and yields of milk and milk components, with increases more evident in cows with higher milk yields, indicating that there was significant variation in response. Reasons why only higher yielding cows responded more positively to stearic acid supplementation than lower yielding cows remains to be determined. In a recent dose response study with mid lactation cows feeding an 85% stearic acid supplement increased DMI but had no effect on the yields of milk or milk components when compared to non-FA supplemented control diet (Boerman and Lock, 2014b).
Interestingly, there is mechanistic data to support the concept that individual FA can impact milk fat synthesis differently. Hansen and Knudsen (1987) utilized an in vitro system and reported that palmitic acid stimulated de novo FA synthesis and incorporation into triglycerides whereas other FA were either neutral or inhibitory (Figure 4). In addition, there were only minor differences in the esterification efficiency into triglycerides of various FA, except for palmitic acid, which was a better substrate than the other FA tested (Figure 4).

Figure 3. Effect of palmitc (C16:0) and stearic (C18:0) acid supplementation on milk fat concentration and yield compared to non-fatty acid supplemented control diets (Steele and Moore, 1968; Steele, 1969).



SUPPLEMENTAL FAT INTERACTIONS WITH OTHER DIETARY COMPONENTS

The composition of the basal diet can also be an important element of production responses to FA supplements. For example, partial substitution of corn silage with another forage such as alfalfa has been shown to negate the negative effect of tallow on milk fat yield (Onetti et al., 2004). In high producing dairy cows an interaction was observed between forgage:concentrate ratio and response to supplemental FA (Weiss and Pinos-Rodriguez, 2009). In high-forage diets increased energy intake from supplemental saturated FA (mixture of palmitic and stearic acids) was directed mostly to body reserves, whereas in low-forage diets the increased energy intake from the saturated FA supplement was directed mostly to milk production. Using lower producing cows Grum et al. (1996) compared diets at 2 different forage:concentrate ratios either without or with added saturated FA (mixture of palmitic and stearic acids). At both forage:concentrate levels supplemental saturated FA increased milk fat concentration and yield, whereas saturated FA supplementation had opposing effects on DMI when supplemented in the low and high forage:concentrate diets (Table 3). van Knegsel et al. (2007) fed either high lipogenic or high glucogenic diets with the same concentrate to forage ratio (40:60). Additional FA in the lipogenic diet were provided by Ca-salts of palm FA distillate and palm oil. Cows fed the lipogenic diet partitioned more energy to milk than cows fed the glucogenic diet and had a higher milk fat yield. No difference were found for energy retained as body protein, but energy mobilized from body fat tended to be higher in cows fed the lipogenic diet (van Knegsel et al., 2007).

Figure 4. Effect of palmitic (C16:0), stearic (C18:0), and oleic (C18:1) acid on lipid biosynthesis in dispersed ruminant mammary gland epithelial cells. Re-drawn from Hansen and Knudsen (1987).

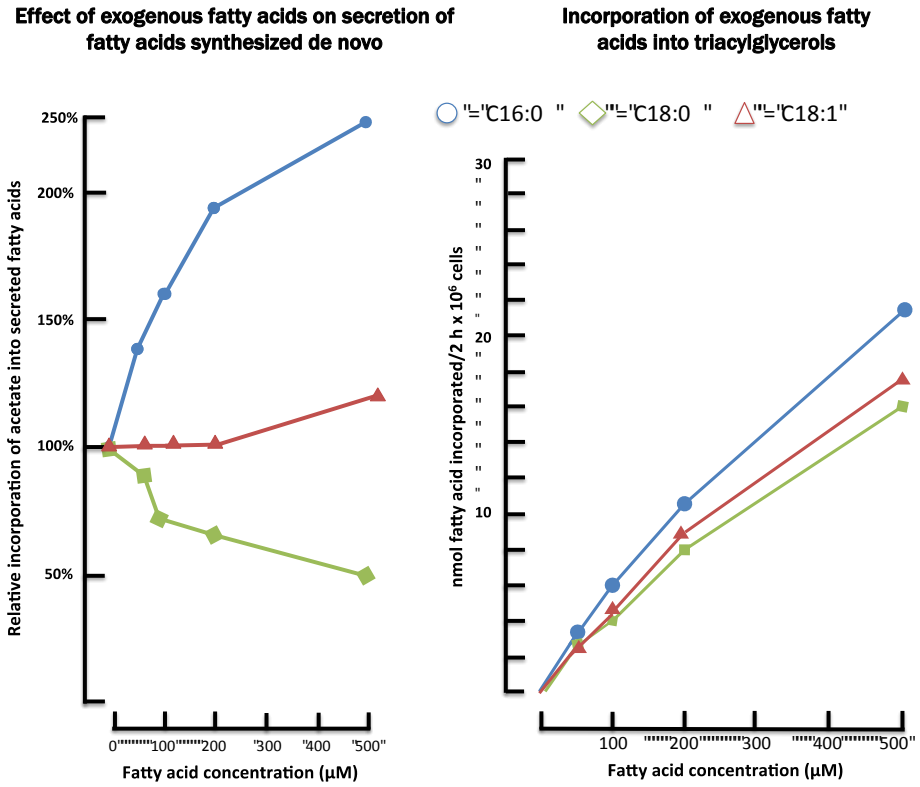


Table 3. Production responses of dairy cows fed increased energy from saturated FA (mixture of palmitic and stearic acids) or concentrate. Adapted from Grum et al. (1996).

	Treatment[1]				
Variable	LC	LC + F	HC	HC + F	SEM
NDF, % DM	32.8	33.6	27.5	28.4	
FA, % DM	2.8	5.7	2.5	5.1	
DMI, kg/d[4]	19.2	20.7	20.2	19.4	0.4
Milk, kg/d	27.3	29.4	28.3	27.8	1.1
Fat, %[2, 3, 5]	3.52	3.83	2.98	3.33	0.11
Fat, kg/d[2, 3, 5]	0.96	1.11	0.83	0.91	0.06
4% FCM, kg/d[3, 5]	25.3	28.5	23.7	24.8	1.4
Protein, kg/d	0.85	0.87	0.92	0.88	0.05

[1]LC: low (45%) concentrate and no supplemental FA; LC + F: low concentrate plus 3% DM supplemental FA; HC: high (70%) concentrate and no supplemental FA; HC + F: high concentrate plus 3% DM supplemental FA. Diets LC + F and HC were isoenergetic (1.7 Mcal/kg).
[2]Significant effect of FA supplementation.
[3]Significant effect of concentrate.
[4]Significant effect of the interaction between FA supplementation and concentrate.
[5] Significant effect of the comparison of equal energy density treatments (LC + F vs. HC).

Clearly, further work is required to characterize the impact of different FA on production responses across different diet types and different levels of production. Of note in Table 3 is the comparison of equal energy density treatments (LC + F vs. HC). Although predicted energy density was similar between these treatments the LC + F treatment increased fat-corrected milk yield. In a recent study using high producing post-peak dairy cows we fed either a high fiber and FA diet (HFF) containing a 50:50 ratio of forage to concentrate containing a palmitic acid-enriched supplement at 2.5% of diet DM or a high starch diet (HS) containing a 40:60 ratio of forage to concentrate (Boerman et al., 2014). The two treatments resulted in similar apparent energy densities and intakes but the HS treatment partitioned more energy toward body gain whereas the HFF treatment partitioned more energy toward milk (Table 4). Whether cows on the high fiber diet would have maintained milk yield similarly to the high starch diet without the inclusion of the palmitic acid-enriched supplement remains to be determined. In established lactation, cows are usually in positive energy balance and the goals are to maximize milk and component yields and reduce excessive conditioning. Further work is necessary, but high fiber and FA diets might diminish the incidence of over conditioning in mid-lactation cows while maintaining high milk production.

Table 4. Body weight, body condition score, and calculated energy values for cows fed a high fiber diet containing a palmitic acid-enriched supplement or a high starch diet containing a mixture of dry ground and high moisture corn (Boerman et al. 2014).

	Treatments[1]			*P*-value[2]
Variable	HFF	HS	SEM	TRT
BW	678	685	14.8	0.01
BCS	3.07	3.20	0.09	< 0.001
Change in BW, kg/d[3]	0.33	0.78	0.10	0.003
Change in BCS, pt/28 d	- 0.01	0.24	0.03	0.001
Calculated energy values[4]				
Apparent NE_L of diet Mcal/kg	1.78	1.79	0.02	0.64
Milk, Mcal/d	32.8	32.6	1.05	0.05
Body Tissue Gain, Mcal/d	1.95	4.90	0.58	0.001
Maintenance, Mcal/d	10.6	10.7	0.17	0.02
Partitioning				
Milk, %	72.8	67.9	1.11	< 0.001
Body Tissue Gain, %	4.03	10.1	1.16	0.001
Maintenance, %	23.2	22.0	0.43	0.01

[1] Treatments were either a high fiber and FA diet (HFF) containing a 50:50 ratio of forage to concentrate containing a palmitic acid-enriched supplement at 2.5% of diet DM or a high starch diet (HS) containing a 40:60 ratio of forage to concentrate containing a mixture of dry ground and high moisture corn.
[2] *P*-value associated with treatment differences (HFF vs. HS; Trt).

SUPPLEMENTAL FATS AND ESSENTIAL FATTY ACIDS

Feeding supplemental FA to aid dairy cow reproduction is of considerable interest at the present time, both to scientists and the agricultural industry. This interest is based on several reasons; first, the well documented reduction in reproductive performance of dairy cows throughout the world has driven the development of nutritional strategies to reverse this trend; second, the use of dietary FA supplements will intensify as nutritionists strive to increase the energy density of diets to meet requirements of the high producing dairy cow; and third we now recognise that FA, both of dietary and rumen origin, can have specific and potent effects on ruminant metabolism. A variety of FA supplements have been tested for their effect on reproductive performance in lactating cows. Supplemental FA often improve pregnancy rates though there is large variability in responses observed; results, however, are rarely negative (Thatcher et al., 2011). Most data indicate that the observed improvements in reproductive parameters are independent of energy balance; therefore, supplemental FA are likely improving reproductive performance via effects of specific FA impacting metabolism and function of the ovary and uterus, rather than simply having a caloric effect. This has resulted in an increased interest in various oil seeds and in designing rumen inert FA sources that will deliver specific unsaturated FA to the lower gut for absorption. Inconsistencies observed in the literature might be explained by variation in the availability of the specific FA for incorporation into uterine tissues as a result of the extensive metabolism of dietary FA in the rumen and/or variation in effects on DMI, energy balance, and energy partitioning.

FA sources enriched in n-6 FA (e.g. most plant oils) or n-3 FA (e.g. linseed oil and fish oil) that deliver these FA to tissues beyond the rumen are of considerable interest from both reproduction and animal health (immune function) standpoints. When considering individual FA and different supplements it is important to realise that different families of FA (e.g. n-6 vs. n-3 FA) most likely impact reproductive or immune function processes via different pathways. For example, n-3 FA have been shown in vitro and in vivo to have a suppressing effect on $PGF_{2\alpha}$ synthesis, whereas linoleic acid (n-6) promotes $PGF_{2\alpha}$ synthesis (Santos et al., 2008). Interestingly, Greco et al. (2015) showed that supplying the same quantity of FA in the diet of early lactation dairy cows but altering the ratio of n-6 to n-3 FA influenced lactation

performance and inflammatory responses to an LPS challenge. In particular, reducing the ratio of n-6 to n-3 FA increased DMI and the yield of milk and milk components. This is a promising area of investigation and more research is needed to better identify the most effective FA sources and specific FA and how best to deliver these to the dairy cow. The amount and type of supplemental FA to be fed and the optimum window for supplementation will depend on the goals of the nutritional strategy employed and on the post-rumen delivery of specific FA from the supplement.

SUMMARY

The addition of supplemental FA sources to diets is a common practice in dairy nutrition to increase dietary energy density and to support milk production. Although in general FA supplementation has been shown to increase milk yield, milk fat yield, and the efficiency of milk production, great variation has been reported in production performance for different FA supplements, and indeed the same supplement across different diets and studies. Further work is required to characterize the sources of variation in response to FA supplementation. Just as we recognize that not all protein sources are the same it is important to remember that not all FA supplements are the same. The key is to know what FA are present in the supplement, particularly FA chain length and their degree of unsaturation. Once this information is known it is important to consider the possible effects of these FA on DMI, rumen metabolism, small intestine digestibility, milk component synthesis in the mammary gland, energy partitioning between the mammary gland and other tissues, and body condition. Interactions with other dietary components and the level of milk production are also important in determining the response to various FA supplements. The extent of these simultaneous changes along with the goal of the nutritional strategy employed will ultimately determine the overall effect of the supplemental FA, and the associated decision regarding their inclusion diets for lactating dairy cows.

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