Does Animal Agriculture Contribute to Antibiotic Resistance in Humans? Current Insights into Dairy Cattle and their Role in Antibiotic Resistance

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▪ Take Home Messages

▸ Antibiotics are important tools for managing disease in dairy cattle.

▸ Bacterial evolution invariably results in some degree of antibiotic resistance.

▸ Prudent use of antibiotics can reduce the risk and extent of antibiotic resistance.

▸ Antibiotics should not be used as a substitute for good management practices.

■ Introduction

The term antibiotic is used to describe antimicrobial agents that are effective against bacteria. Antibiotics are used in dairy cattle production primarily to treat or prevent disease and to a lesser extent to increase milk production or improve feed efficiency. Thus, antibiotic use in dairy production can be classified as therapeutic when used to treat an existing disease condition, prophylactic when administered during periods of high disease risk and sub-therapeutic when administered to enhance production.

Antibiotics kill or inhibit the growth of bacteria. No antibiotic is completely effective at inhibiting all target bacteria within the complex microbial communities that are frequently encountered in agricultural systems. Consequently, it is inevitable that antibiotic therapy will eventually reduce the

number of antibiotic-susceptible strains and promote the development of antibiotic-resistant strains. Feeding antibiotics at sub-therapeutic dosages can promote antibiotic resistance as more bacteria may survive the antibiotic challenge and the duration of exposure is often prolonged. Nontherapeutic use of antibiotics, and sub-therapeutic use in particular, is coming under increasing scrutiny by policy-makers, scientists and the general public. Their concerns arise mainly from the possibility that antibiotic-resistant bacteria may be transferred from livestock to humans, through animal to human contact, through the environment (e.g., water, manure) or in contaminated food products (e.g., meat, milk).

Although it is widely accepted that using antibiotics in livestock production can lead to development of resistant bacteria, the risk that this poses to humans is less clear. At present, the scarcity of information on this relationship, and the complexity of the events associated with animal to human transfer, make it challenging to predict the true risk to human health. Several European countries have already implemented legislation restricting the use of antimicrobial agents in animal agriculture. Health Canada has recently announced efforts to promote the judicious use of medically important antimicrobial drugs in food animal production by removing claims of growth promotion and/or production and increasing the oversight of veterinarians for antimicrobial use in food animals (Health Canada, 2014). This paper provides an update of current knowledge on the development of bacterial resistance to antibiotics as it pertains to antibiotic use in dairy production. Recommendations will also be made for prudent use of antibiotics to minimize development of antibiotic-resistant bacteria.

■ Antibiotic Use in Dairy Cattle

Administration of antibiotics to dairy cattle is usually therapeutic, that is, in response to development of symptoms of disease. This type of chemotherapy shortens the period of antibiotic administration and usually reduces the total amount of antibiotic employed. If label recommendations are followed, the dose is high enough to kill or inhibit the target bacteria and the risk of resistance is minimized. If resistance does develop, it is likely to be short term, because the genetic cost of maintaining the resistance trait reduces the competitiveness of resistant bacteria once antibiotic therapy ceases. Thus, in the absence of the chemical challenge, the resistant population is gradually replaced by antibiotic-susceptible bacteria (Figure 1). However, there are instances in which antibiotics are administered prophylactically (e.g., dry cow infusion, medicated milk replacer) and sub-therapeutically (e.g., ionophores, sulfonamides) to dairy cattle. Long term, low doses of antibiotics are more likely to produce antibiotic-resistant bacteria (Salyers, 1999). In this situation, the antibiotic concentration is low enough for continued bacterial growth, but high enough to exert a selective pressure favoring the establishment of resistant bacteria. Antibiotics however, are not the only driver for selection.

Often antibiotic resistance genes can be located on mobile genetic elements that also confer other fitness traits, such as virulence and resistance to metals. Consequently, selective pressure for these other traits can result in the co-selection and maintenance of antibiotic resistance genes in the absence of antibiotic selection. Selection and maintenance of antibiotic resistance genes in bacteria is therefore not simply 'black and white'.
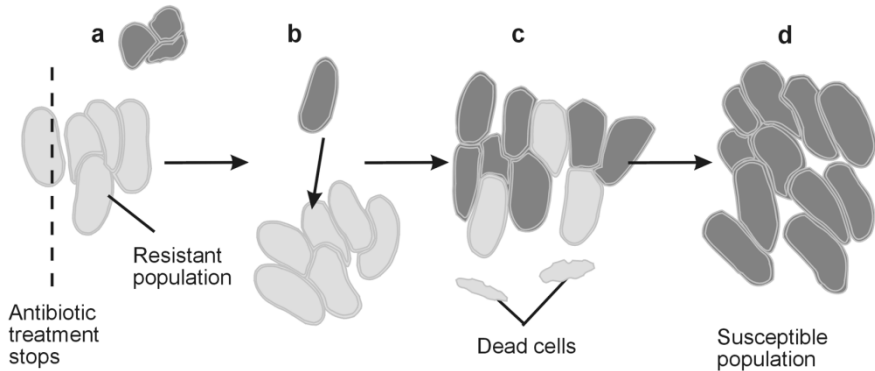

Figure 1. Steps involved in the transition from an antibiotic-resistant bacterial population to an antibiotic-susceptible population once antibiotic therapy has ceased. a) In the presence of the antibiotic the bacterial population consists of resistant cells and some susceptible cells that may have entered a dormant state or survived the antibiotic therapy. b) In the absence of the selective pressure of the antibiotic, susceptible cells may exit dormancy and enter a viable state. c) Eventually susceptible cells start to dominate the population as they may have a competitive advantage over resistant cells in the absence of selective pressure from the antibiotic. d) Eventually the population is dominated by susceptible cells, but there are usually a few resistant cells that persist and will proliferate should the selective pressure of the antibiotic return.

Antibiotics inhibit the growth of, or kill target bacteria by a variety of mechanisms (Table 1). Many antibiotics inhibit the process of protein synthesis, thereby preventing the bacterium from producing the various enzymes and structural proteins required for survival. Other antibiotics interfere with the synthesis of the bacterial cell wall or destabilize the ionic gradients that are required for substrate transport and cellular energetics. An antibiotic's effectiveness is greatly dependent upon the physiology of the target bacterium. Thus, using an antibiotic against bacteria for which it was not designed will not only fail to control the disease, but will also increase the likelihood that other non-target bacteria will develop resistance. Moreover, antibiotics are completely ineffective against viruses and their use in this

manner increases the likelihood that bacterial resistance will develop. Consequently, correct identification of the causative agent of the disease and strict adherence to antibiotic label recommendations is one of the easiest ways of reducing the development of antibiotic resistance in bacteria. In addition, withdrawal times are also indicated on labels as a strategy to reduce the amount of antibiotic residues in meat and milk for the purposes of food safety (Table 1).

Table 1. Examples of common antibiotics and antimicrobial agents administered to dairy cattle

Antibiotic family (Source)	e.g. Trade names	Target-action	Withdrawal times*	
			Milk	Meat
Aminoglycosides (*Micromonospora* spp., *Streptomyces* spp.)				
Gentamicin	Gentamicin	Primarily Gram negative, Inhibit protein synthesis	-	-
Cephalosporins (*Cephalosporium acremonium*)				
Ceftiofur hydrochloride	Excenel	Inhibit cell wall synthesis,	-	4d
Cephapirin	Metricure Sus	Broad spectrum activity	0hr	48hr
Ionophores (*Streptomyces* spp.)				
Monensin	Rumensin	Primarily Gram positive, Interferes with ion	-	0d
Lasalocid	Bovatec	transport	Non-lac	0d
Macrolides (*Streptomyces* spp.)				
Tilmicosin	Micotil	Primarily Gram positive,	Non-lac	28d
Erythromycin	Erythro-36	Inhibit peptide bond	36hr	14d
Tylosin	Tylan	formation	Non-lac	21d
Penicillins (*Penicillium* spp.)				
Penicillin G	Formula 17900 Dry-Clox	Inhibit cell wall synthesis	72hr	15d 30d
Cloaxcillin	Orbenin Quick Release		Non-lac	28d
Ampicillin	Polyflex sterile		48hr	6d
Tetracyclines (*Streptomyces* spp.)				
Chlorotetracycline	Aureomycin	Broad spectrum, Inhibit	Non-lac	24hr
Oxytetracycline HCl	Liquamycin	protein synthesis	Non-lac	15-28d
Others				
Florfenicol	Nuflor		Non-lac	28d IM; 38d SQ
		Broad spectrum, Inhibits bacterial protein synthesis		
Novobiocin	Albadry	Broad spectrum, Inhibits protein and nucleic acid synthesis	72hr	30d
Pilimycin HCl	Pirsue	Primarily Gram positive, Inhibits protein synthesis	36hr	21d
Antibacterials				
Trimethoprim/Sulfadoxine	Borgal	Broad spectrum, Inhibit thymidine synthesis	96hr	10d
Sulfamethazine	AS-700	Broad spectrum, Inhibit folic acid synthesis	-	7d

*Withdrawal times based on FDA guidelines; Non-lac, non-lactating cattle

In dairy cattle, antibiotics are used to treat a variety of bacterial diseases (Table 2). The first recorded use of antibiotics in dairy cattle was for the treatment of mastitis (Foley et al., 1946) and this disease still accounts for the majority of antibiotic use in dairy production. Despite the widespread use of antibiotics for over 50 years, mastitis is an extremely common disease in most dairies. This attests to the fact that antibiotics cannot be used to eradicate disease-causing bacteria. Rather, they can be used to mediate the disease condition, but the bacteria responsible for the disease will undoubtedly

continue to persist within the environment. Formation of biofilms is one of the strategies employed by bacteria to persist in the environment and can facilitate the survival of antibiotic-resistant bacteria (Marchand et al., 2012). Antibiotics are a valuable tool for controlling infections, but they will remain so only if they are used in a manner that does not promote the development of bacterial resistance. Bacteria are naturally opportunistic and when environmental (e.g., poor hygiene) or physiological conditions (e.g., depressed immunity, nutritional stress) favor their growth, it is inevitable that the disease condition will once again be expressed.

Table 2. Common bacterial targets of antibiotics and antimicrobials in dairy cattle

Condition	Causative bacteria
Common	
Bovine Respiratory Disease (Pneumonia)	*Mannheimia haemolytica*
	Pasteurella multocida
	Histophilus somni
	Mycoplasma bovis
Enteric disease (Diarrhea)	*Escherichia coli*
	Clostridium perfringes
	Salmonella spp.
Mastitis	*Staphylococcus aureus*
	Streptococcus agalactiae
	Streptococcus spp. (environment)
	Klebsiella/E. coli/Enterobacter
	Pseudomonas spp.
	Actinomyces pyrogenes
Foot rot	*Fusobacterium necrophorum*
	Bacteroides nodosus
Metritis (Uterine infection)	*Actinomyces pyrogenes*
	Fusobacterium necrophorum
	Bacteroides spp.
Ocular (Pink eye)	*Moraxella bovis*
Less common	
Lumpy jaw	*Actinomyces bovis*
Listeriosis	*Listeria* spp.
Anaplasmosis	*Anaplama marginale*
Tetanus, blackleg	*Clostridium* spp.
Wooden tongue	*Actinobacillus lignieresii*

■ Mechanisms of Antimicrobial Resistance

Gene Evolution and Transfer

As with the rest of the natural world, bacteria are in a state of continuous evolution. Unlike complex organisms such as cattle or humans, bacteria have exceedingly short life cycles and entirely new generations can be produced in a matter of hours or days. Consequently, the opportunity for intergenerational evolution in bacteria is far greater than it is in higher life forms. Furthermore, bacteria exist in the environment in unimaginable numbers. For example, there are more bacteria in a cubic centimeter (cc) of rumen fluid (10 billion) than there are people on earth. Thus, the likelihood that one individual bacterium will express a unique genetic trait is far greater than with organisms that exist in far lower numbers.

Bacteria have also evolved several mechanisms of exchanging genetic material (Figure 2; Levy, 1992). If the genetic material codes for a trait that confers resistance to a particular antibiotic, then there is a significant likelihood that recipient bacteria will become resistant to that same antibiotic. Resistance genes are exchanged via three main routes: conjugation, transduction and transformation (Wozniak et al., 2010). Conjugation is the process through which plasmids are exchanged between bacteria. Resistance genes are frequently carried on plasmids, which are loops of DNA that readily undergo both intra- and inter-species transfer. Transduction is the process whereby bacteria can become infected with viruses (i.e., bacteriophage) that pick up antibiotic resistance genes and transfer them during the infection of other bacteria. Finally, transformation involves the uptake of 'free DNA' that can code for antibiotic resistance from adjacent bacteria that have died and underwent cell lysis. Integration of resistance genes, acquired through transduction or transformation, into the chromosome or plasmids is required for these genes to become functional. In many cases, these segments of genetic material have specialized properties that promote chromosomal integration, often introducing whole families of resistant genes in a single transfer event (Bass et al., 1999).

Integrative conjugative elements (ICE) are a form of mobile genetic element (MGE) that have gained much interest in the last couple of years. Unlike other MGE, ICE are self-transmissible as they encode all the machinery required for them to excise from the chromosome, circularize and replicate to a new host through conjugation (Wozniak et al., 2010). ICE have been identified in both gram-positive and gram-negative bacteria, with many occupying a wide host range (Wozniak et al., 2010). ICE can carry genes coding for resistance against many antibiotics. For example, our lab isolated bacteria that cause pneumonia in cattle that were resistant to 11 different antibiotics (Klima et al., 2014). The ability of ICE to carry multiple resistance genes and transfer to a

wide host range makes them an important vehicle in horizontal gene transfer (HGT). Although knowledge of ICE in dairy cattle is limited, the prospect of these mobile elements to alter bacteria from being killed by antibiotics to being resistant to almost all antibiotics used for treating pneumonia in cattle is unnerving.
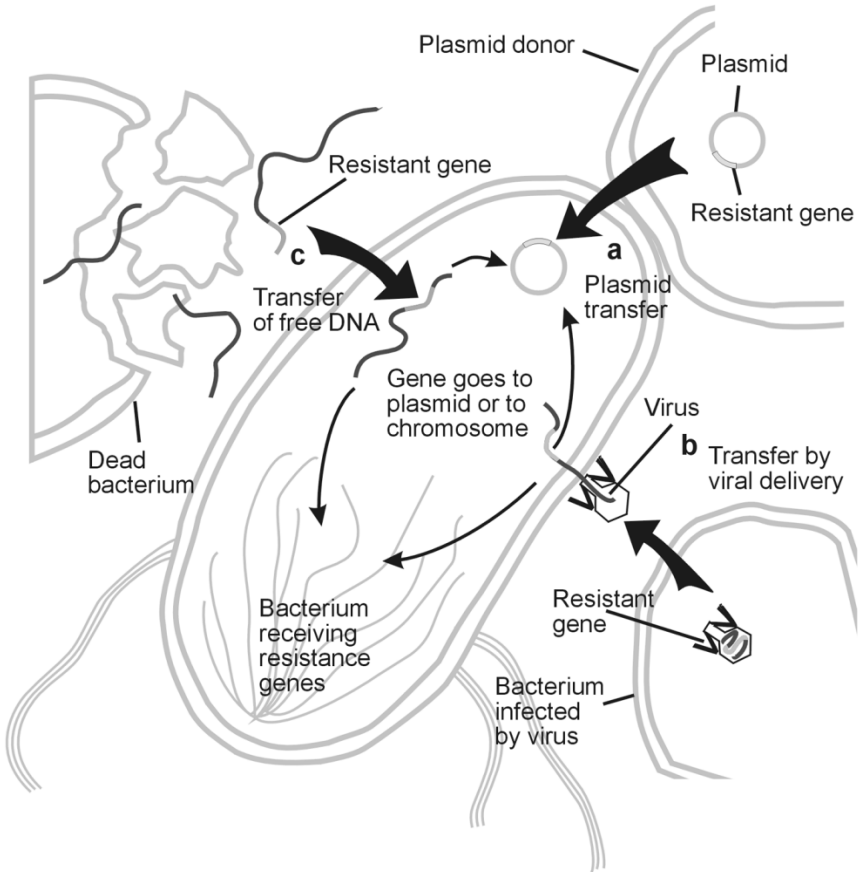

Figure 2. Mechanisms of gene transfer in bacteria, including a) transfer of plasmid from another bacterial cell; b) transfer via viral carrier; c) uptake of free DNA released from another cell.

Mechanisms of Antibiotic Resistance

Bacteria have a myriad of resistance mechanisms that can be employed to render an antibiotic ineffective (Figure 3). One of the most common mechanisms of resistance is the production of enzymes that degrade the antibiotic (Davies, 1994). For example, hydrolysis of the four-membered β-lactam ring by β-lactamase is largely responsible for widespread resistance to penicillin. Alternatively, by altering their cell surface, bacteria can effectively reduce the affinity of a drug for its target site (Spratt, 1994). In some cases, bacteria develop antibiotic efflux mechanisms, which rapidly pump the antibiotic out of the cell before it has a chance to interfere with cellular processes. This is apparently the mechanism of resistance employed by *Salmonella typhimurium* against the antibiotic florfenicol, the active ingredient in Neuflor®.
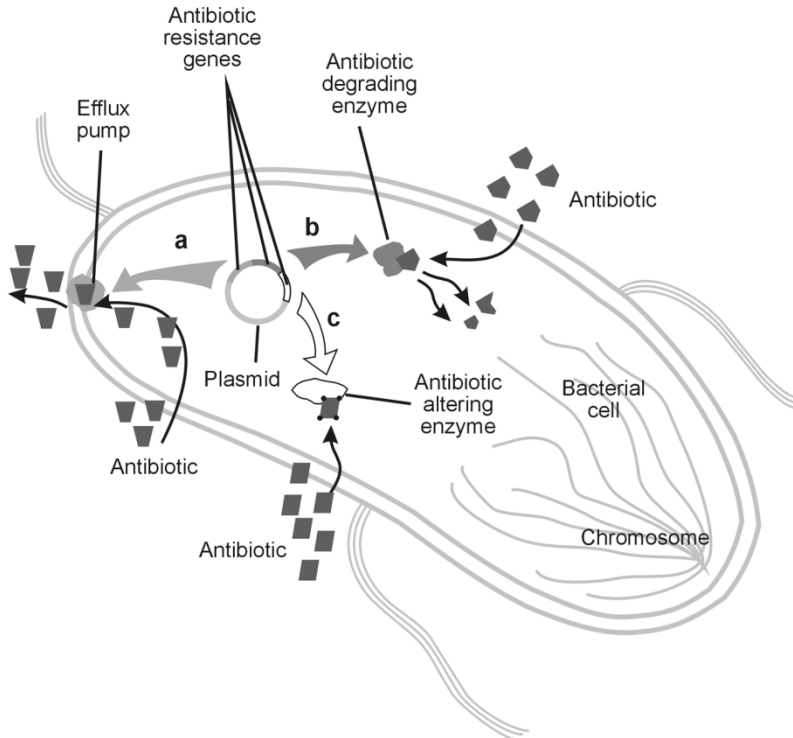

Figure 3. Examples of methods in which bacteria inactivate antibiotics, including a) rapid removal of the antibiotic from the cell prior to cellular damage; b) production of an enzyme which degrades the antibiotic; c) inactivation of the antibiotic through attachment of additional chemical groups.

In other cases, bacteria produce specific enzymes that attach additional chemical structures onto the antibiotic, thereby rendering it inactive. For example, O-phosphorylation of the antibiotic erythromycin has been observed in a number of bacterial isolates (O'Hara et al., 1989). In one of the more complicated mechanisms of resistance, a bacterium will develop metabolic bypasses to override the biochemical reaction that the antibiotic is designed to inhibit. This type of mechanism confers resistance to the antibacterial agent trimethoprim (Davies, 1994). In yet another tactic, bacteria may simply overproduce the targeted metabolic product, thereby overwhelming the amount of antibiotic that has been administered. This method of resistance is employed against sulfonamides and trimethoprim.

Bacteria may also resist antibiotics by forming biofilms. Biofilms can form on material commonly found in the milk processing environment, including rubber and stainless steel (Suarez et al., 1992). Biofilms are complex microbial communities that limit the interaction of antibiotics with bacterial cells and also provide an environment that promotes exchange of genetic material among cells (Licht et al., 1999). In biofilms, bacterial cells are encased in a secreted exopolysaccharide matrix that also entraps metabolic byproducts which may serve as secondary substrates. Bacterial biofilms play an important role in the dairy herd health as well as food hygiene, being one of the main recontamination sources of milk (Marchand et al., 2012). Examples of biofilm-related diseases include chronic mastitis (*Staphylococcus* spp. and *Streptococcus* spp.) and chronic pneumonia (*Pasteurella* spp. and *Actinomyces* spp.). Because they are resistant to removal by antibiotics and biocides, organisms employing this growth form represent a potential source of chronic infection if not properly controlled, explaining why some mastitis infections are so difficult to control.

The mechanisms of this biofilm resistance to antibiotics are not clearly understood but are most likely multi-factorial, involving uptake of the drug by the microorganism, inhibition of diffusion of the antibiotic through the biofilm, and alterations in bacterial metabolism. Table 3 illustrates the differences in susceptibility to antibiotics of biofilm- and free form- (planktonic) bacterial isolates from clinical mastitis cases. The minimum inhibitory concentration (MIC, ug/ml) is the concentration of drug necessary to prevent the growth of planktonic bacteria, the standard method of measuring the sensitivity of bacteria to antibiotics. The minimum biofilm eradication concentration (MBEC) was proposed to describe the concentration of a particular antibiotic or biocide necessary to eliminate bacteria growing on a surface as a biofilm. Undoubtedly, the MBEC value more closely represents the effective dose in a clinical situation (Ceri et al., 1999). Killing bacteria associated with biofilms may require concentrations of antibiotic thousands of times greater than those required to kill bacteria floating freely in a fluid environment. Moreover, the use of an ineffective antibiotic or biocide to control biofilm bacteria may lead to the development of genetic resistance in planktonic forms of bacteria.

Microbial biofilms are often composed of multiple species of microorganisms, which can mutually protect one another against biocidal products during sanitation making elimination more difficult (Vlkova et al., 2008). For example, streptococci form predominantly monospecies biofilms whereas *Pseudomonas* spp. are more likely to produce multispecies biofilms, hence sheltering other spoilage or pathogenic bacteria and allowing them to persist (Marchand et al., 2012). Adequate sanitization procedures are therefore important for effective biofilm control. Sanitation generally involves the sequential use of caustic and acid wash steps, and the procedure varies depending on the equipment being cleaned. Application of sanitizers may also be included in the cleaning process. Continued research into alternative agents and strategies for biofilm control, such as using enzymes and ultrasonic cleaning, are being investigated (Marchand et al., 2012).

Table 3. Comparison of the antibiotic sensitivity of free floating (planktonic) and adherent (biofilm) bacteria

Antibiotic	*Staph. aureus*		*Strep. uberis*		*E. coli*		*Klebsiella*	
	MIC	MBEC	MIC	MBEC	MIC	MBEC	MIC	MBEC
Amikacin	<2	4	8	8	4	16	4	8
Gentamicin	<2	4	<2	<2	<2	4	<2	<2
Tilmicosin	<2	1024	4	1024	128	>1024	512	1024
Pirlimycin	4	>1024	<2	64	1024	>1024	1024	>1024
Cephalothin	<2	1024	<2	128	16	256	16	64
Erythromycin	<2	512	<2	32	64	>1024	256	512
Penicillin G	512	>1024	<2	256	512	512	256	1024
Novobiocin	<2	256	<2	>1024	128	128	128	>1024
Tylosin	<2	1024	<2	512	1024	>1024	512	1024
Cloxacillin	<2	512	<2	512	512	512	1024	36914
Cepthapirin	<2	1024	<2	32	64	128	16	32
Oxy-tetracycline	<2	256	<2	128	256	256	<2	8
Ceftiofur	<2	1024	<2	128	<2	<2	<2	8
Enrofloxacin	<2	64	<2	2	<2	<2	<2	<2
PenG/Novo	<2	512	<2	64	256	1024	256	>1024

■ Evidence that Antibiotic Use in Dairy Farms is Increasing Antibiotic Resistance

Comparison of amounts of antibiotic resistant bacteria on organic dairy farms, where antibiotics are infrequently used, to conventional dairy farms, where antibiotics are employed, may offer insight into the possibility that antibiotic use in dairy farms is increasing resistance. Studies comparing antibiotic susceptibility of *S. aureus* and other bacteria involved in mastitis have reported mixed results. Some have indicated greater susceptibility to antibiotics in organic compared to conventional dairy farms (Tikofsky et al., 2003), or only for certain antibiotics (Sato et al., 2004b), whereas others have indicated little difference in the amount of antibiotic resistant bacteria between organic and conventional dairies (Roesch et al., 2007). A number of bacteria have been examined, including *Escherichia coli*, *Campylobacter* spp. and

Salmonella spp. and in some instances little difference was found between conventional and organic dairies (Ray et al., 2006; Sato et al., 2004a). Other studies have reported greater resistance with conventional dairies (Halbert et al., 2006; Sato et al., 2005). Consequently, it is not clear if conventional production practices are leading to increased resistance as results vary depending on the type of bacteria and antibiotic examined. The general consensus from these studies is that most bacteria from both production systems remain susceptible to most antibiotics (Halbert et al., 2006; Tikofsky et al., 2003).

■ Risk of Antibiotic Use in Dairy Farms Impacting on Human Health

There is an increasing trend for people to consume raw (unpasteurized) milk and milk products (Oliver et al., 2009). This increases the risk of exposure to foodborne pathogens, and the incidence of illness and disease in humans. Even of greater concern is the possibility of exposure to multidrug-resistant pathogens through the consumption of contaminated raw milk and milk products. There have been a number of cases where the consumption of raw milk products has been linked to infection with multidrug-resistant *Salmonella* (Cody et al., 1999; Villar et al., 1999). General quality control practices such as pasteurization kill these pathogens and minimize multidrug-resistant pathogens. There is also a risk that milk can become contaminated with multi-drug resistant pathogens after pasteurization. A multidrug-resistant *Salmonella enterica* serotype Typhimurium was linked to an outbreak caused by adulterated milk in Pennsylvania and New Jersey (Olsen et al., 2004). However, if proper hygienic practices are employed exposure to multidrug-resistant pathogens can largely be avoided.

Application of manure to agriculture fields as well as leakage of manure lagoons are other points of concern. Not only does manure introduce bacteria carrying antibiotic resistance genes into the environment, but it can also expose bacterial populations in fields and potentially in water to residual antibiotics (Heuer et al., 2011). Transfer of resistance genes from bacteria in manure to indigenous soil bacteria can promote the persistence of resistance genes in soil (Heuer et al., 2011). Srinivasan et al. (2008) found there was a greater distribution of multiple resistance genes in bacteria isolated from dairy farm soil regularly applied with cow manure compared to nondairy soil with no known history of exposure to manure from animal agriculture. They also found that some bacteria carried class 1 integrons, a form of MGE located on transposons, suggesting the possibility that these bacteria are able to acquire and disseminate resistance genes to other bacteria (Srinivasan et al., 2008). Sequencing of the bacterial DNA in dairy cow manure showed that it contained many novel and diverse antibiotic resistance genes (Wichmann et al., 2014).

Evidence of manure as a potential contributor to the resistance problem suggests that more focus should be placed on how it is managed. Composting of livestock and poultry manure decreases residual antibiotics in manure (Dolliver et al., 2008; Selvam et al., 2012) and reduces the amounts of antibiotic resistant and pathogenic bacteria (Edrington et al. 2009; Sharma et al. 2009). However, antibiotic resistance genes can persist even in composted manure (Sharma et al., 2009). Survival of bacteria carrying resistance determinants can therefore facilitate HGT with soil bacteria. Although composting is an effective practice at reducing residual antibiotics, it is not successful at eliminating all antibiotic resistant bacteria. Factors such as compost temperature and duration may influence the survival of these bacteria.

Application of livestock manure containing residual antibiotics is known to alter the composition of the soil bacterial community. Recently, Udikovic-Kolic et al. (2014) reported that application of manure from dairy cattle that did not receive antibiotics increased the resistance of resident soil bacteria to beta-lactamases. Beta-lactamase resistance was the focus of this study, but it is possible that manure may enrich for other resistant bacteria present in the soil. Many bacteria (e.g., *Penicillium* spp., *Streptomyces* spp., *Micromonospora* spp. and *Bacillus* spp.) naturally produce antibiotics which kill or inhibit the growth of competing bacteria. In fact, many of the antibiotics used in dairy production originated from these bacteria (Table 1). vIt is therefore not surprising that resistance was able to be detected in soil bacteria despite the fact no antibiotics were administered to the dairy herd.

■ Strategies for Addressing Antimicrobial Resistance

Concerns over the impact of the use of antibiotics in food producing animals first arose in 1969 after the release of the Swann report by the Joint Committee on the use of Antibiotics in Animal Husbandry and Veterinary Medicine. Fears of cross-resistance to vancomycin from the feed additive avoparcin resulted in the European Union (EU) banning its use in 1997. Since 2006, the EU has banned the use of all antibiotics for growth promotion (Capita and Alonso-Calleja, 2013). A number of surveillance programs have been established by various European countries. One of the most extensive monitoring schemes is the European Antimicrobial Resistance Surveillance Network (EARS-Net) which has participation from all 28 EU member states and two European Economic Area (EEA) countries. This surveillance program mainly focuses on human isolates; however, other smaller surveillance programs, such as DANMAP in Denmark and NORM/NORM-VET in Norway, focus on a collection of isolates from both humans and food-producing animals.

In North America, there are movements to begin to phase out the use of

certain antibiotics for enhanced food production. In the USA, the FDA released a guidance document in 2012 on 'The Judicious Use of Medically Important Antimicrobial Drugs in Food-Producing Animals'. The purpose of this document is to provide recommendations regarding the appropriate or judicious use of medically important antimicrobial drugs. Medically important drugs are those that are considered important for the treatment of human diseases. Recommendations include limiting medically important antimicrobial drugs to the prevention or treatment of disease and not for growth promotion, and an increase in veterinary oversight and the requirement for consultation (FDA, 2013). This is a voluntary plan that should reduce the use of antibiotics considered important in human medicine in livestock and poultry production. Canada is following a similar approach to the USA (Health Canada, 2014). Surveillance of antibiotic resistance is carried out in Canada and the USA and examines isolates from humans and food production animals to encompass a 'one health' approach for monitoring resistance.

With recent movements to phase out the use of certain antibiotics in North America, antibiotics that are commonly used in dairy production may no longer be available for use in the same capacity as in the past (Table 1). This is even more reason for farmers to ensure they employ good management practices to minimize the need to use antibotics. Third generation cephalosporins, macrolides (erythromycin), trimethoprim/sulfonamides, and fluoroquinolones are classified by the FDA as critically important antibiotics for human medicine (FDA, 2003). These antibiotics are currently used in dairy production and may be the first to be reassessed in terms of their use (Table 1). Others that fall into the highly important category, including aminoglycosides, penicillins and aminopenicillins, may be the next to be targeted. Prudent use of antibiotics maybe essential if some of these antibiotics are to continue to be available for use in dairy production.

■ Keys to Prudent Antibiotic Use

The key to prudent use of antibiotics in livestock production is to use the right antibiotic at the right time in the right manner. A few of the key points to keep in mind are listed below:

‣ Do not use antibiotics to compensate for poor nutrition, poor hygiene, or the lack of immunization or implementation of a herd health program.

‣ Consider other methods of intervention (e.g., proper nutrition, stress management) prior to antibiotic therapy.

‣ Use antibiotics in consultation with a veterinarian.

‣ Avoid extra-label use of an antibiotic if possible. If considered absolutely necessary, extra-label use should be done in consultation with a veterinarian and in accordance with government regulations.

▸ Select dosing rates and treatment periods in accordance with manufacturers' recommendations. "Cutting" or administering a dose lower than what is recommended will increase the likelihood of resistance and reduce the effectiveness of the antibiotic.

▸ Minimize as much as possible the use of antibiotics considered important for treating human disease.

▸ Select narrow spectrum antibiotics on the basis of their target organism(s), not on their withdrawal time.

▸ Whenever practical, culture suspected pathogens for identification to ensure that the selected antibiotic is targeting the causative organism.

▸ Limit the use of antibiotics to ill or high-risk animals; minimize the number of animals treated as much as possible.

▸ Maintain accurate treatment records and select the antibiotics that are most effective for your operation.

▸ Ensure that antibiotics are properly stored and handled, and dispose of them correctly once their expiry date has passed.

■ Conclusion

Bacteria are a natural and essential component of the environment. Using antibiotics to declare "all-out war" against bacteria is a war that we cannot win. In fact, heightened use of antibiotics has the potential to reduce, rather than increase, our ability to control disease-causing bacteria. Instead, antibiotics must be used with the precision of a surgeon's knife, being employed strategically against target bacteria, and only as one component of an overall herd health management program. Failure to use antibiotics with respect could lead to their eventual elimination as a tool in animal production, either through regulatory restrictions or through the loss of their effectiveness due to the emergence of resistant bacterial populations. It is important to remember that the individuals most likely to come into first contact with antibiotic-resistant bacteria in the dairy are the dairy producers and their families.

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Avoiding Silage Problems

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▪ Take Home Messages

- Preservation of silage depends on the combination of low pH, fermentation acids and the absence of oxygen.

- Design silos for a minimum feed out rate of 30 cm/day from the whole face.

- For ensiling in bunkers, piles or bags, ensile between 30 and 40% dry matter (DM) for most crops except legume forages (35 to 40% DM).

- Pack bunkers and piles to achieve a minimum bulk density of 700 kg/m^3.

- Seal silos with a high quality plastic film in one or two layers, whether an oxygen barrier film or thick polyethylene film.

- Plastic film needs to be held tightly to the silage surface to prevent it from acting as a bellows drawing oxygen under the plastic.

- Plastic laid down the walls of bunker silos can reduce or eliminate shoulder spoilage.

- Seal edges and joints in plastic with continuous weighting material (gravel filled bags, piles of soil, etc.)

- Use a defacer for unloading bunkers or piles to improve DM recovery by 1 or more percentage points by making a smooth face.

- When ensiling forages that could become clostridial, ensile them separately using a homo-fermentative lactic acid bacterial inoculant and then feed after 2 to 4 weeks of storage.

- When heating issues are common, review silage management first to see if density, sealing and feed out rates are contributing to the problem and correct those issues first. If silo design and management are good, using a chemical additive or a *Lactobacillus buchneri* inoculant at ensiling can help keep silages stable.

▪ Silage Basics

The goal in ensiling a crop is to keep its nutritional value similar to that of the crop at ensiling. If we are to realize that goal, we need to understand how ensiling preserves the crop; then, all of the recommended steps in making high quality silage will make sense. Knowledge of preservation principles helps you troubleshoot when silage quality turns out poorer than expected.

The ensiling process is straightforward. We put a crop in a structure, pile, bag or wrapped bale so we can exclude oxygen. In the absence of oxygen, lactic acid bacteria ferment crop sugars to primarily lactic acid as well as acetic acid, ethanol and other compounds. The acids lower crop pH. This fermentation may last a few days to a month or more depending on temperature and the dry matter (DM) content of the crop.

Preservation of the crop is dependent on 3 factors: low pH, the acids, and the anaerobic (oxygen-free) environment. The 3 contribute in different ways. When one is removed or not sufficient, silage quality may be compromised.

Low pH is the primary means of preventing the growth of clostridia bacteria. Clostridia produce butyric acid from sugars and lactic acid and ferment amino acids to ammonia and amines. Significant clostridial activity (typically butyric acid levels >0.5% DM) reduces intake and predisposes cows to ketosis. The necessary pH to prevent clostridia from growing depends on the DM content of the silage (Figure 1). A lower pH is needed in low DM silages to stop clostridia.
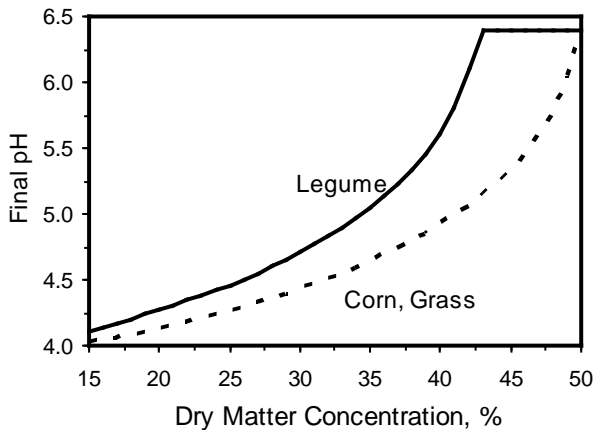

Figure 1. The pH below which growth of *Clostridium tyrobutyricum* ceases (Leibensperger and Pitt, 1987).

Lactic acid and acetic acid not only lower pH but can inhibit the growth of some microorganisms. High lactic acid concentrations (>5% DM) help prevent *Listeria monocytogenes* (cause of listeriosis in cattle and humans) in silage from growing, as well as inhibiting some spoilage bacteria. High acetic acid is effective at inhibiting yeasts and molds that spoil silages in the presence of oxygen. Acetic acid is the primary means by which inoculants containing *Lactobacillus buchneri* improve aerobic stability.

While acetic acid is good for slowing yeast and mold growth, rarely is the combination of high acetic acid concentration and low pH sufficient to prevent all spoilage microorganisms from growing. Spoilage microorganisms (whether yeasts, molds or bacteria) require oxygen to grow; so, keeping oxygen out of the silo by an effective seal is the only means of completely keeping them in check. When the silo is opened, oxygen can readily diffuse through the open face and spoilage microorganisms begin to grow potentially increasing losses, reducing silage quality, and in the worst cases, heating the silage.

▪ Silo Design

The first step, and one of the most important for avoiding silage problems, is to have a properly designed silo. Current recommendations from the University of Wisconsin are to design bunkers or piles so that a minimum of 30 cm of silage are removed from the whole face each day. When using bags, you will want to take more than 30 cm/d. These high feed out rates are recommended because there is enough oxygen 1 m back from the face of a well-packed silo to allow spoilage microorganisms to grow at full speed based on studies in Germany, Israel and the U.S. At the former recommendations of 15 cm/d, spoilage microorganisms have a week to grow before the silage is in the feed bunk. As shown in Figure 2, losses during feed out go up dramatically at low feed out rates because you are increasing the time the silage is exposed to oxygen prior to the cattle eating the silage.

Tools are available under the Harvesting and Storage section (http://fyi.uwex.edu/forage/harvest/) of the University of Wisconsin Team Forage website to help in the proper design of a bunker, pile or bag silos. To use these, you need to know the number of livestock that are being fed and the expected inclusion rate of that silage in their rations. These numbers along with estimated silage density permit a range of solutions leading to feed out rates at or above the 30 cm/day recommendation.
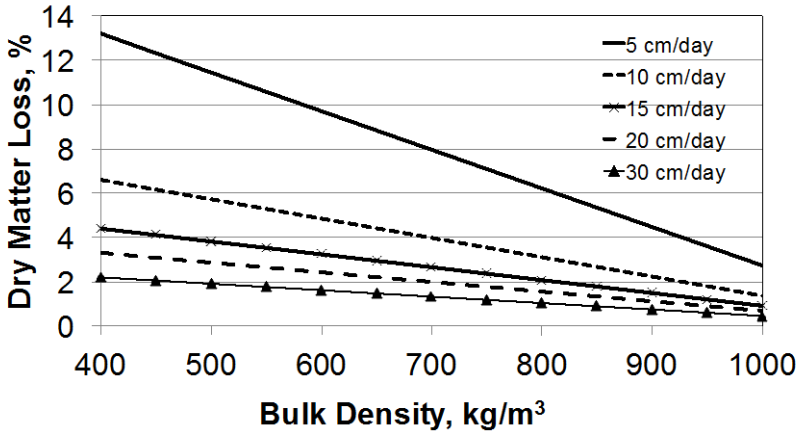
Figure 2. Simulated dry matter losses during bunker silo emptying as a function of bulk density and feed out rate based on Pitt and Muck (1993).

▪ Harvest at the Right DM Content

Ensiling at the right DM content helps to avoid a variety of silage problems. For horizontal silos, the recommended range for best ensiling results is 30 to 40% DM. Why that particular range? At less than 30% DM, there are 2 major risks. One risk is silage effluent or seepage, which is a loss of soluble nutrients from the silage, and is far more environmentally damaging than manure slurry. With tall bunkers or piles (>5 m high at the peak), you may need to ensile several points drier than 30% to avoid effluent and avalanches.

The second risk of ensiling too wet is clostridial fermentation. As indicated in Figure 1, the lower the DM content, the greater the amount of fermentation and the lower the pH needed to avoid clostridial fermentation. The level of risk depends on the crop. The risk is low with corn silage, which has a high sugar content and low buffering capacity resisting pH drop. The risk is intermediate with most grass and small grain silages. The risk is highest with legume silages like alfalfa. In Wisconsin, we typically recommend 35% DM as the minimum DM content for ensiling alfalfa because our environmental conditions make it difficult to get silage pH below 4.7; so, ensiling drier is necessary to avoid clostridial fermentation. While clostridial fermentation is rarely a problem when ensiling corn silage at less than 30% DM, there has certainly been anecdotal evidence of abnormal fermentations when corn is ensiled too wet. Also, you may be losing starch content in harvesting corn at less than optimum maturity (half to three-quarters milk line).

Ensiling too dry increases the potential for aerobic spoilage. This is discussed in more detail under packing, but a drier crop is more difficult to pack and achieve a low porosity (i.e., the fraction of volume in the silo filled with gas

surrounding silage particles) that limits oxygen movement into silage from the open face or an opening in the cover.

The other major silo types have higher optimum DM ranges. With wrapped bales, the best results are from ensiling forages between 40 and 60% DM. Long forage particles do not ensile as well as chopped forage so a higher range is needed to avoid clostridial fermentation. Tower silos need drier forage to avoid effluent and also for optimum material handling.

▪ Particle Size

A range of theoretical lengths of cut is readily achievable on most forage harvesters today. Also, various types of kernel processors are commonplace for harvesting whole-crop corn. Is there a best particle size setting to avoid silage problems? Unfortunately there is not an easy answer. From an engineering perspective, small particle size (e.g., 10 mm theoretical length of cut) is best for creating a high density in the silo, which in turn limits oxygen movement into the silo and thus DM losses. However, ruminant livestock need their ration to contain some long fiber for good rumen function. If the ration contains sufficient long hay, then silage particle size is not that important and can be set low. If silages are the only sources of forage in the ration, then harvesting at a longer theoretical particle size (e.g., 20 mm) may be necessary for good livestock health.

With whole-crop grain silages, cracking the kernels is an important factor for efficient utilization of the starch. This is usually accomplished by a kernel processor on the forage harvester. Because kernel processors reduce particle size, the theoretical length of cut for the harvester is usually set higher (e.g., 20 mm). Kernel processing score for corn silage (percentage of starch passing through a 4.75 mm sieve) is now available from forage testing labs in the U.S. to assess how well kernels have been broken. Optimum scores for kernel processing in corn are values above 70%. Kernel processing is important for utilization of the starch in the silage by livestock, and not to avoid a silage management problem.

▪ Packing

The packing of bunker or pile silos may have a substantial impact on losses by minimizing the porosity, the principal factor governing the movement of oxygen into the silo whether during feed out or if there is damage to the cover. To keep porosity at approximately 40% or less (a reasonable target), you need to achieve a bulk or as-fed density of at least 700 kg/m^3.

How do you achieve high densities in bunkers or piles? Research carried out in Wisconsin by Dr. Brian Holmes (University of Wisconsin-Madison) and me

indicates the most important factors are packing tractor weight, packing time/tonne of crop, crop moisture and spreading layer thickness. Density increases with heavier tractors, more packing time per tonne, higher crop moisture and spreading each load over a larger area in the silo. Taller bunkers and piles will achieve higher densities than lower ones. However, height should be restricted to the height that your unloading equipment can reach for safety reasons. Drier crops take greater effort to achieve a high bulk density. When packing piles, all slopes (sides, back, front) should be no greater than 1:3 height:length. This helps achieve a high density while providing for safe packing side-to-side as well as front-to-back. In addition, these low slopes allow for a tight seal between the cover and silage, which will minimize losses, especially during feed out.

We developed 2 spreadsheets to improve silage density (Bunker Silo Density Calculator, Silage Pile Density Calculator), and these are available on the UW Team Forage website under the Harvest and Storage section (http://fyi.uwex.edu/forage/harvest/). These spreadsheets allow you to estimate how changes in your packing practices affect density. As harvest rate increases, achieving a high density may require more than one packing tractor, and using tractors of similar weight maximizes the effect of adding more packing tractors. If a second tractor is less than half the weight of the first tractor, then the additional tractor provides little benefit. If you have a high harvest rate but small bunkers, filling 2 bunkers simultaneously with one packing tractor in each may be an alternative.

Packing bag silos properly is an art. The adjustment of density varies by the model of bagging machine. The goal is to produce as high a density as possible while maintaining a smooth bag. This is easier with corn silage than with alfalfa. A lumpy bag allows oxygen to move easily back from the face when feeding and makes the silage more susceptible to spoilage losses in warm weather.

▪ Sealing

Spoiled silage at the top of a bunker or pile is common. Unfortunately, it presents producers with a real dilemma. Do you feed the spoilage and risk the health of your herd from potentially feeding mycotoxins, listeria or other pathogens, or do you pay for the removal and risk the safety of the farmhand who removes the spoiled silage? The best alternative is to do such a good job of sealing the silo that there is no spoiled silage. There are 3 critical components to eliminating top spoilage: the quality of the plastic film, how well the joints and edges are sealed, and how well the film is held against the crop.

Let us begin with the type of plastic. Today, the only good solution for covering a silo or bale is plastic. In the past, plastic meant polyethylene, and

there were different thicknesses and colors. Today, oxygen barrier films and polyethylene cling films are also available. The oxygen barrier films typically are a sandwich with an inner layer of film that is very resistant to oxygen movement covered on both sides with polyethylene.

So what film should you use to cover your bunker, pile, bag or bale? That is a difficult question to answer because no researchers have looked at all of the types and combinations of products on the market. Some researchers have done trials but looked for answers at the silo face rather than immediately under the plastic films; their results suggest there isn't much difference between films when in fact there can be substantial differences. When evaluating the research, it is very important to know how it was conducted, and in some cases it is not clear. So I am going to stick to studies we have done. These are studies done on real bunkers where we have compared the quality of the forage in the top 60 cm under the film before and after ensiling but really most of the difference is in the 15 cm immediately below the film.

Our initial studies compared 6 mil (6/1000") black polyethylene with 8.5 mil white/black polyethylene. With our various comparisons, color did not make any significant differences but thickness did. There was on average a 5-percentage point improvement in DM recovery in the top 15 cm using 8.5 mil vs. 6 mil polyethylene.

Several years later we compared the original 2 mil Silostop oxygen barrier film covered by a woven tarp to 8.5 mil polyethylene. We applied the polyethylene using our standard practice at the time, which meant that the plastic was just on top with tires and tire sidewalls completely covering the surface. The plastic was cut to lap up the wall to minimize rain entering the silage and held in place by tires. The Silostop film was used according to the manufacturer's system, which meant Silostop was placed on the bunker walls, and the tops of those sheets folded onto the top of the bunker. A top sheet of Silostop was laid over the whole top and then covered with a woven tarp secured with gravel bags. The Silostop system clearly outperformed our standard system near the walls (cores taken 60 cm away) with DM losses in the top 15 cm being reduced by 15 percentage points. The higher losses near the walls are attributable to the inadequate seal at the shoulder with the standard practice. In the middle of the sheets, DM losses were not significantly different between the 2 systems; however, pH and silage fermentation products from cores in the middle of the sheets (Table 1) did show lower pH and higher lactic acid to acetic acid ratios under Silostop, indicating less oxygen exposure under Silostop.

	Depth, cm	pH	Lactic Acid	Acetic Acid	L:A
Haylage					
White	0-15	4.89	2.5	4.0	0.6
Silostop	0-15	4.82	4.5	2.2	2.1
White	15-30	4.82	4.5	1.7	2.6
Silostop	15-30	4.75	3.8	1.4	2.7
Corn					
White	0-15	4.02	3.2	1.6	2.0
Silostop	0-15	3.98	3.0	1.2	2.6
White	15-30	4.00	4.1	1.4	2.9
Silostop	15-30	3.97	3.9	1.2	3.1

Table 1. Average silage pH and fermentation acid concentrations (% DM) in silage immediately beneath the center of 8.5 mil white polyethylene or Silostop oxygen barrier film in bunker silo trials at the U.S. Dairy Forage Research Center.

We have another comparison that adds to the picture. We compared Raven Industry's FeedFresh product, an oxygen barrier film, with their standard 5 mil polyethylene film in 2 bunker silos. On average, the FeedFresh product reduced DM losses by 8 percentage points in the top 15 cm.

Some people believe the original Silostop oxygen barrier film worked so well because of its clinginess, and today one sees thin polyethylene films that are clingy to be used as an underlayment. We have not studied these, but multiple layers of polyethylene will create a thicker barrier that should result in reduced losses whether or not the clinginess property is of any value.

Overall, research shows oxygen barrier films provide the lowest DM losses visually, indicating no spoilage has occurred. Polyethylene can perform similarly if you use an 8.5 mil product; however, 8.5 mil polyethylene is not common as a bunker cover. I would speculate most producers are using 4 to 6 mil products at a cost in DM losses of 10 to 5 percentage points, respectively, in the top 15 cm under great management.

Quality of film is not the full answer to preventing spoiled silage at the top. Sheets need to be overlapped sufficiently (at least 1 m), and the overlaps and edges secured with gravel bags or tires butted together. More than that, if any film is billowing in the wind, it can act like a bellows drawing in air around the edges of the sheet and permitting spoilage. To prevent this, the standard tires-touching-tires keep the plastic in place. Today a number of tarps (woven or expanded mesh) secured with gravel bags are available that can do a similar job of keeping the plastic tight to the silage. These tarps need to be reused for multiple years to be cost effective. In snowy places like Wisconsin, it is best to have narrow tarps laid parallel to the feed out face for easier removal during winter.

A particular problem with bunker silos is shoulder spoilage at the walls. This is caused by air infiltrating between the wall and the cover plastic as well as rain running off the plastic and through the silage at the wall. An effective way of eliminating shoulder spoilage is to place plastic down the walls prior to filling. These side wall sheets should lap onto the forage top at least 1 m at the end of filling, be covered by the cover sheet and then secured by gravel bags or tires butted together. Side wall film not only eliminates shoulder spoilage, it also limits oxygen penetration through concrete and cracks in the concrete and keeps silage acids from etching the concrete walls, extending their life.

At bunker ends or the edges of piles, it is important to have a tight seal with the ground or pad. Extend at least 1 m of plastic onto the ground or pad, and provide a continuous weighting on that film. Weighting can be soil, sand, gravel or gravel bags butted together. These typically will produce better results than tires.

Finally, inspect the plastic weekly and repair holes that develop during storage using tape designed for the film used. This is especially true for silo bags and wrapped bales as well.

▪ Feed Out

Once you open a silo for emptying, oxygen can move into the silage at the face. The exposure of the silage to oxygen and the subsequent DM losses are regulated by feed out rate as indicated in Figure 2, as well as by the porosity of the silage achieved in the packing process. By the time you get to emptying a silo, you have little ability to alter these factors, except for possibly increasing the number of animals or the amount of silage per animal being fed.

What you can do to minimize losses during feed out is to keep a smooth face and not leave piles of loose silage at the bottom of the face. The value of using a defacer versus bucket on a skid-steer or tractor provides a small but significant benefit in improved DM recovery (Figure 3). The benefit is greater if density and/or feed out rate are low. However, even under excellent conditions a defacer can pay for itself.
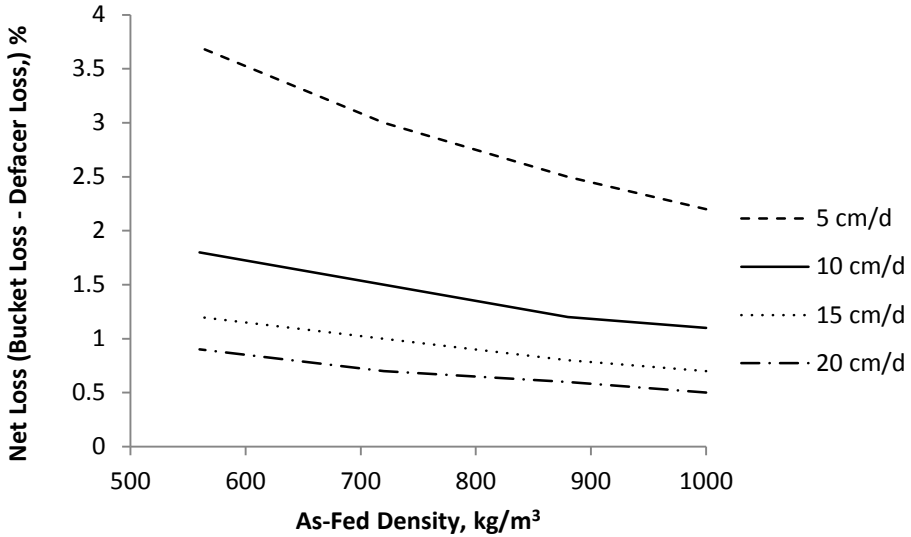
Figure 3. Net improvement in dry matter recovery by using a defacer as affected by bulk density and feed out rate (Muck and Rotz, 1996).

▪ Additives

It is not possible to provide a full discussion of silage additives here. However, additives may be tools to avoid some silage problems. There are 2 issues where silage additives may be of benefit: avoiding clostridial fermentation and reducing the potential for spoiling/heating silage.

Homo-fermentative lactic acid bacteria are the most common silage additives in North America. These bacteria supplement the natural population of lactic acid bacteria on the crop and help guarantee a fast, efficient fermentation in the silo. These inoculant bacteria produce primarily lactic acid and few other products. Because lactic acid is a strong acid, the inoculant helps guarantee the lowest possible pH from silage fermentation. They may be beneficial if ensiling a grass or legume silage that is a bit wetter than is recommended and there is the potential for the silage to turn clostridial.

Heating of corn silage in the feed bunk or at the silo face is a common problem during the summer. If this is a common problem for you, the first step is to review your silage management. High dry matter content, covering issues, low densities and low feed out rates are the most common sources of these problems and should be addressed before looking to silage additives. However, it is possible to be doing well in all these areas and still have some

summer issues. Two classes of additives may be helpful: chemical additives and *Lactobacillus buchneri*. *Lactobacillus buchneri* slowly converts lactic acid to acetic acid, which inhibits yeasts and molds. Typically it takes 1.5–2 months of storage for a substantial effect. For silages that will be fed after a short ensiling time, a chemical additive may be more effective. Chemical additives include propionic acid, propionic-acetic acid mixtures, potassium sorbate and sodium benzoate. With all of these additives, one must be sure the application rate is at or above the label directions and the product is well mixed with the forage for these products to be effective in preventing heating.

▪ Potential Problems

Potential Clostridial Problems

Invariably there will be times when forage is ensiled too wet in order to avoid rain on wilted forage. In such circumstances, there are several things one can do to minimize the risk of having clostridial silage. One, do not layer that wet forage in with dryer forage in a bunker or pile. It will only potentially create a clostridial layer between good layers that will be difficult to separate. It is better to create a bag or a mini-pile of the wet forage that is ensiled separately. Two, use a good homo-fermentative inoculant to get pH as low as possible. Do not use an inoculant with *Lactobacillus buchneri* in the formulation. Three, feed this silage out as early as possible. The best situation is to allow it to ferment and stabilize for 2 to 4 weeks and then start feeding. Why? It usually takes months for a silage to go clostridial. So, by feeding out early, you may be able to completely feed the silage before it goes clostridial.

Potential Heating Problems

If you are beginning to see heating when feeding a particular silage, there are several things you can do to possibly minimize it: 1) Make sure you are very fastidious in leaving a smooth face on the silo and there is no loose silage left on the floor after feeding is done; 2) Minimize the amount of exposed silage on the top of the bunker or pile and keep tires or gravel bags butted against each other across the edge of the cover; 3) Consider increasing the number of feedings per day or increasing the number of animals fed from the silo; 4) If heating cannot be prevented, then application of propionic acid or a propionic/acetic acid mixture to the TMR may be needed to keep the ration cool in the feed bunk.

Visible Mold

Visibly moldy silage is greatly reduced in nutritional quality from what it was when placed in the silo. It may contain mycotoxins, bacterial toxins, and pathogens such as *Listeria monocytogenes*. Some silage nearby the moldy

layer may be clostridial with butyric acid and amines. This material should be discarded because of its potential to adversely affect herd health. More importantly it should cause you to review your management because improved practices can avoid moldy silage.

▪ Acknowledgements and Disclaimer

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Feeding the Fresh Cow: What is the Ideal Carbohydrate Mix?

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▪ Take Home Messages

▸ Based on field observations and limited research, fresh cows should be housed in small, separate groups to minimize social stress, maximize comfort of the physical resting space, provide a feeding area to minimize slug feeding and other undesirable feeding behaviors, and provide a diet that promotes dry matter intake and prevents health problems.

▸ The nutritional strategy that is used during the transition period of cows is critical for supporting lactation performance while minimizing the risk of ruminal acidosis, controlling inflammation, and improving metabolic health. Thus, the fresh diet should be formulated in the context of the dry and high group diets to provide a smooth nutrient change from one diet to the next.

▸ The fresh diet should contain an appropriate blend of fermentable carbohydrates (i.e. starch, sugar, and fibre) to contribute to the energy demands of the cows while maintaining the integrity of the rumen epithelium and rumen health.

▸ The risk of subacute ruminal acidosis and chronic inflammation increases when higher starch fresh diets are fed, especially following lower starch, controlled energy dry cow diets.

▸ The amount of physically effective fibre that is needed in the fresh diet is influenced by the rumen fermentable starch content of the diet and the dry matter intake of the cow. The role of undigestible fibre in the fresh diet is being defined.

▪ Introduction

Feeding and management practices for transition dairy cows can have a substantial impact on a cow's well-being and farm's profitability. Suboptimal

transitions from the dry cow diet to the early lactation cow diet can decrease milk yield, lactation persistency, and reproductive performance. The use of a fresh cow diet can make the transition more successful.

▪ The Fresh Pen

The use of a fresh pen continues to grow in popularity, especially for dairies that are expanding herd size and/or building new facilities. A fresh pen allows a dairy to house fresh cows separately from other cows in the lactating herd to facilitate monitoring of health problems, minimize social stress, and provide a diet specifically formulated for fresh cows.

The optimal duration for cows to remain in a fresh pen is unknown but likely is unique for each dairy and possibly each cow given differences in rate of increase in dry matter intake (DMI) and milk production. An informal survey of dairies suggested that cows remain in a fresh pen anywhere from 10 to 42 days in milk (DIM) with 14 to 21 DIM the most common. Fresh cows that transition successfully are typically ready to move to a high group pen with a more fermentable carbohydrate diet between 10 and 14 DIM. Extended stays in a fresh pen can limit DMI because of gut fill, and increase the risk of health problems, such as primary ketosis. An example of this occurred at Miner Institute where the primary forage in the fresh diet, corn silage, had a lower fibre digestibility than expected based on initial laboratory analysis. Cows increased intake rapidly until 10 to 14 DIM when intake plateaued with milk continuing to increase. The cows were eating as much fibre as a percentage of their body weight as possible. Blood beta-hydroxybutyrate (BHBA) started to rise at a time when it would normally decrease resulting in some cows having subclinical ketosis or showing clinical signs of ketosis. At 22 DIM, cows were switched to a more digestible high group diet that allowed greater intake and the primary ketosis problem resolved.

A fresh pen and its management can greatly influence fresh cow behavior. A fresh pen typically houses a smaller group of cows together than the other lactating groups, which reduces the social activity and possibly leads to less social stress and more resting. This concept was demonstrated in a study (Burow et al., 2009) where the addition of fresh cows to small groups of cows compared to large groups of cows housed at 1 stall per cow resulted in fewer agonistic and non-agonistic interactions within the 3 hours after mixing. Introducing fresh heifers as pairs rather than individuals to a group containing older cows promoted lying behavior after mixing (O'Connell et al., 2008). In another study (Østergaard et al., 2010), cows housed as a separate group for one month after calving with ≥1 stall per cow resulted in improved production and health in primiparous but not multiparous cows. Interestingly, a fresh cow diet was not used in the separate group. An additional benefit of separate grouping may be observed if an appropriate fresh cow diet is used.

The feed bunk of a fresh pen should be understocked and provide at least 76 cm of space or ≥1 headlock per cow. Limited feed buck space increased the number of displacements and feeding rates of cows before and after calving (Proudfoot et al., 2009). Fresh cows that were overcrowded at the feed bunk altered their feeding behavior (e.g. increased feeding rate) and increased the risk for health problems associated with slug-feeding (Krawczel et al., 2009).

Based on field observations and limited research, fresh cows should be housed in small, separate groups to minimize social stress, maximize comfort of the physical resting space, optimize size of the feeding space to minimize slug feeding and other undesirable feeding behaviors, and provide a diet that promotes DMI and prevents health problems.

▪ Fresh Cow Feeding Strategies

There are many studies that have evaluated the carryover effects of dry cow diets on metabolism and performance during early lactation. However, fewer studies have evaluated nutritional strategies immediately after calving to support the metabolic adaptations and performance (e.g. lactation and reproduction) of the fresh cow. In general, early lactation feeding strategies have focused on increasing the dietary energy density, altering the source of fermentable carbohydrates, and changing the availability of glucogenic nutrients relative to lipogenic nutrients (Dann and Nelson, 2011). Many of the fresh cow feeding recommendations are based on field experience and limited research. Typically, the fresh diet has less forage and more fermentable carbohydrates than the dry diet, but the fresh diet does not have as much fermentable carbohydrate as the high diet. Often the fresh diet is a modified high group diet. Common fresh diet adjustments relative to the high group diet include: increasing the fibre content while decreasing the starch content, including ≤1 kg of straw or hay for more physically effective neutral detergent fibre (peNDF), increasing the rumen undegradable protein content to improve metabolizable protein supply, and having targeted inclusion of other nutrients or additives such as rumen inert fat, yeast or yeast culture, rumen-protected choline, and monensin (McCarthy et al., 2015a).

▪ Source of Fermentable Carbohydrates

The optimal dietary concentration of fermentable carbohydrates (i.e. starch, sugar, and fiber) is being refined for early lactation cows, in particular for fresh cows. Fresh cows need to rapidly increase DMI to support lactation performance while maintaining health. Allen et al. (2009) suggested that liver energy status (i.e. oxidation of fuels such as fatty acids, propionate, lactate, and amino acids) is a major controller of DMI in dairy cows. They suggest that limiting dietary starch content and starch fermentabilty may increase DMI during the fresh period since there will be less rapid production and

absorption of propionate. Another reason, and possibly a more important reason to limit dietary starch and its fermentability in the fresh period is to minimize the risk of subacute ruminal acidosis (SARA), especially when cows transition from a low starch, controlled energy dry diet. More fermentable carbohydrates (i.e. starch, nonforage fiber sources, and highly digestible forages) should be fed to the cows as lactation proceeds and plasma nonesterified fatty acids (NEFA) and BHBA concentrations decrease, as this is a time period when the lower fermentable fresh diet could limit DMI because of gut fill.

Starch

Dietary starch content and fermentabilty in the fresh period (21-day period) has been summarized (McCarthy et al., 2015a; Table 1) recently for 3 studies conducted at Miner Institute (Dann and Nelson, 2011; Williams et al., 2015) and Cornell University (McCarthy et al., 2015b,c).

Table 1. Comparison of dry and lactation diets from Miner Institute and Cornell studies on varying starch levels in the fresh diet.

Study	Starch, % DM	Fermentable starch, % DM	Fermentable CHO, % DM	Starch/CHO, %
Dann & Nelson, 2011				
Dry	13.5	11.5	39.4	29.7
Low fresh	21.0	16.8	42.4	40.1
High fresh	25.5	20.2	44.1	50.3
McCarthy et al., 2015b,c				
Close-up	17.4	15.3	42.2	36.3
Low fresh	21.5	16.8	39.9	42.1
High fresh	26.2	21.5	40.4	53.2

Dann and Nelson (2011) showed that lactation performance was better when cows transitioned from a 40-day dry controlled energy diet (13.5% starch) to early lactation diets containing either 21% starch (fed for 91 DIM) or 23% starch (fed for 21 DIM) followed by 26% starch (fed for 22 to 91 DIM) compared with 26% starch (fed for 91 DIM). The low starch and step-up starch approaches were effective dietary strategies. In contrast, McCarthy et al. (2015a,b) revealed faster rise of intake and milk production when cows were fed a diet containing 26% compared with 21% starch up to 21 DIM. All cows were fed 26% from 22 to 63 DIM. Interestingly, cows were fed a 17.4% starch diet during the close-up period. Perhaps the difference in starch content between dry and fresh diets may be more important than specific dietary starch content fed to fresh cows. It is likely that the large differences in starch content and fermentability between the dry diet and the high starch fresh diet in the Dann and Nelson (2011) study compromised the transition of cows onto the high starch diet in that study. Likewise, feeding the higher starch close-up diet to cows in the McCarthy et al. (2015b,c) study facilitated the transition onto the higher starch fresh diet. Interestingly, the intake of

starch and fiber was lower in the McCarthy et al. (2015b,c) study than the Dann and Nelson (2011) study during early lactation. Given that the fibre digestibility of the diets used in the McCarthy et al. (2015b,c) study were lower than the diets used in the Dann and Nelson (2011) study, it is possible that the McCarthy et al. (2015b,c) cows fed both the low and high starch diets containing 11.5% straw were limited by gut fill during the first 3 weeks after calving. This reinforces the need to use highly digestible fiber sources when lower starch diets are fed.

To better understand the mechanism responsible for the poor transition in the Dann and Nelson (2011) study from a low starch, controlled energy dry diet to a high starch fresh diet, Williams et al. (2015) transitioned cows from a controlled-energy close-up diet (15.5% starch) to 1 of 2 fresh cow diets fed for 21 DIM that varied in starch content (21% vs. 27%) by replacing ground corn with a mixture of soybean hulls and wheat middlings. Measured ruminal pH (Figure 1), ruminal lipopolysaccharide, and serum acute phase proteins (Figure 2) demonstrated that the risk of SARA and inflammation increased with a greater change in dietary starch content and fermentability.
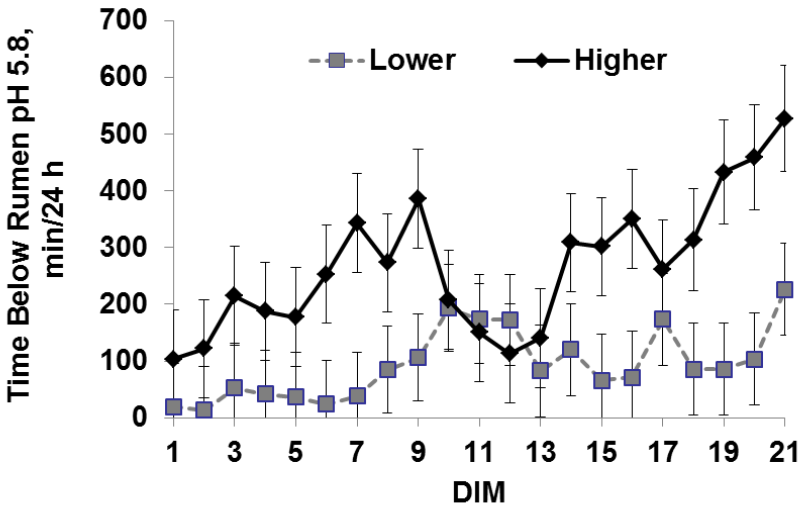

Figure 1. Minutes per 24 hours when rumen pH was <5.8 for cows fed either lower (21%) or higher (27%) starch diets during the first 21 DIM.
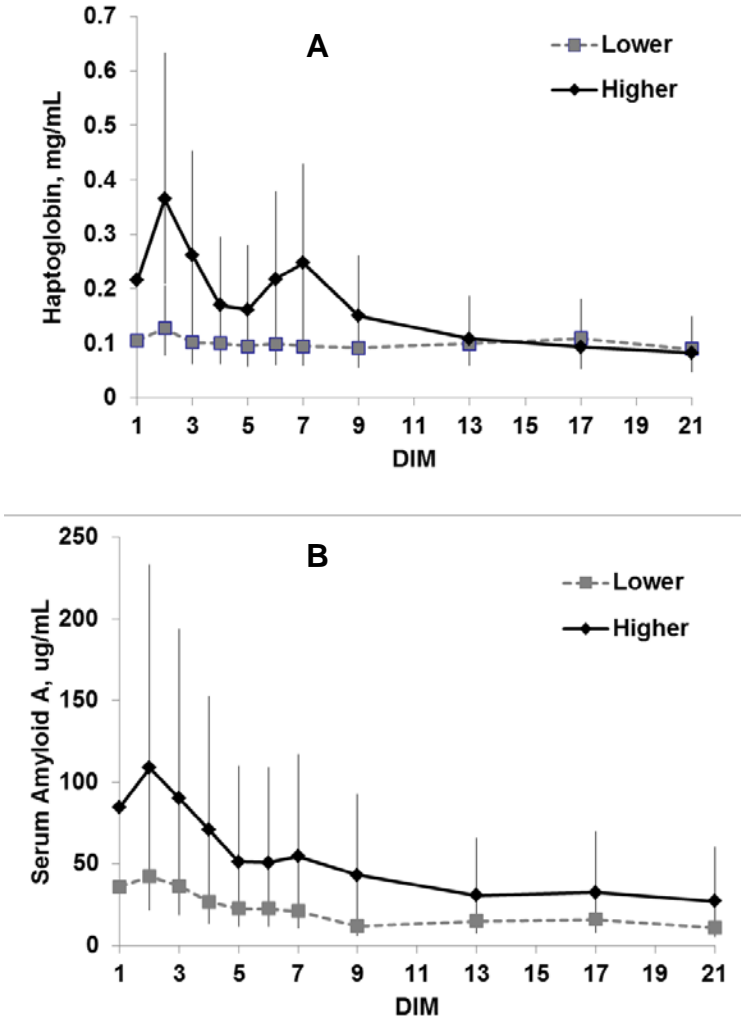
Figure 2. Serum haptoglobin concentration (A) and serum amyloid A (B) for cows fed either lower (21%) or higher (27%) starch diets during the first 21 DIM. (Error bars indicate 95% confidence interval).

Sugar

Sugar ferments faster than starch or fibre in the rumen. However, the rapid fermentation of sugar when it replaces starch in the diet does not typically decrease rumen pH. In mid-lactation cows, additional sugar often increases DMI. Thus, a fresh cow study (Penner and Oba, 2009) attempted to maximize DMI and minimize the risk of ruminal acidosis by partially replacing cracked

corn grain with sucrose in barley silage-based diets. Cows that were fed the higher sugar diet (8.7% sugar; 18.5% starch) had more DMI and milk fat yield, but lower plasma glucose and increased plasma NEFA and BHBA than cows fed the lower sugar diet (4.5% sugar; 20.6% starch). The higher sugar diet reduced the severity of rumen acidosis. In alfalfa silage-based diets, replacement of ground corn with 1.5% sucrose caused a transient increase in DMI during the first 14 DIM, but did not affect DMI or milk yield over the first 84 DIM (Nombekela and Murphy, 1995).

Fibre

Fibre affects intake, digestibility, passage, and rumen function in part because it is less fermentable than starch and sugar. In addition, both the chemical and physical form of fibre is important in maintaining rumen function through rumen mat formation, rumen buffering, and stimulation of rumination. Thus, attention is given to the physical form of the forage ingredients and method of mixing the diet. Physically effective neutral detergent fibre (peNDF) is needed to prevent a severe or extended period of low rumen pH in the fresh diet. However, the amount of peNDF needed depends on the content of the rumen fermentable starch or total starch content of the diet and the amount of DMI (Zebeli et al., 2015). Too much peNDF in the diet will be problematic since it will limit DMI because of gut fill and exacerbate the negative energy balance that occurs during the fresh period. A challenge with the use of peNDF in ration formulation is the methodology and definitions used to determine the requirement.

In recent years there has been renewed interest in the role of undigestible fibre (uNDF) with improved laboratory methodology and modeling capabilities. The evaluation and formulation of uNDF in the fresh diet may be just as critical as peNDF (McCarthy et al., 2015a). Undigestible fibre is important for determining the fast and slow fibre pools and their associated rates of digestion along with estimating gut fill maxima and minima. Observations at Miner Institute suggest that dry and lactating cows eat between ~0.26 to 0.41% of body weight as uNDF. Intake becomes gut fill limited as uNDF nears 0.4% of body weight. A Cornell case study (McCarthy et al., 2015a) found that cows had fewer health problems during the fresh period when theywere fed a diet that resulted in a uNDF intake of ~0.36% of body weight vs. ~0.27% of body weight. The addition of straw or other forages with a high uNDF content to the fresh diet is an easy way to increase the uNDF content. Additional research is needed to determine the optimal level of uNDF in the fresh diet. The optimal level is likely associated with rumen fermentable starch, peNDF, and DMI.

▪ Subacute Ruminal Acidosis in Fresh Cows

Fresh cows are susceptible to metabolic disorders and compromised rumen function during the transition period. A common strategy to reduce metabolic disorders, such as ketosis and fatty liver, associated with the negative energy balance after calvingl is to provide more fermentable carbohydrates in the fresh diet relative to the dry diet. However, large changes in dietary composition and DMI during the transition period increase the susceptibility of cows to SARA. Subacute ruminal acidosis is characterized by repeated bouts of low ruminal pH (<5.8). Bouts can last for several minutes or several hours. The bouts that last >3 hours can negatively affect the ability of ruminal epithelium to absorb volatile fatty acids and decrease fibre digestion through changes in the microbial population. Signs of SARA are often varied and ambiguous, but can include decreased or fluctuating intake, decreased cud chewing, inconsistent manure ranging from stiff to loose, high cull rates due to vague health problems, milk fat depression, poor milk production, and lameness.

Interestingly, SARA and poor rumen health have been identified as causing inflammation (Zebeli and Metzler-Zebeli, 2012; Zebeli et al., 2015). Low ruminal pH can result in the death and lysis of gram-negative bacteria that are in the rumen thereby increasing the free bacterial endotoxin, lipopolysaccharide (LPS), in the rumen. Normally, the epithelium of the rumen acts as a barrier to prevent LPS entry into the blood circulation or the lymphatic system. The acidic ruminal environment, changes in osmotic pressure, and ruminal LPS can damage the epithelium and allow the LPS to translocate into the bloodstream. The presence of LPS in the bloodstream stimulates an acute phase response that results in the production of pro-inflammatory cytokines, acute phase proteins, and systemic inflammation. The activation of the acute phase response is viewed as a protective reaction to reestablish the disturbed homeostasis. However, the presence of inflammation over long periods may be associated with negative consequences for the cow, especially the fresh cow. Prolonged systemic inflammation can 1) cause significant changes in the energy and lipid metabolism, 2) lead to the development of refractory states associated with immune suppression and increased susceptibility to various diseases, and 3) increase the cow's requirements in energy and nutrients, thereby lowering the efficiency of energy and feed use by the cow (Zebeli and Metzler-Zebeli, 2012).

The characterization of SARA and development of feeding strategies for its prevention have been the focus of research for many years (Zebeli and Metzler-Zebeli, 2012; Zebeli et al., 2015). However, most of the research has been focused on mid-lactation cows with little attention given to fresh cows. One study (Penner et al., 2007) with transition heifers found the incidence and

severity of ruminal acidosis increased immediately after calving, emphasizing the need to develop and implement feeding strategies that reduce the risk of SARA. Williams et al. (2015) found that the risk of SARA could be reduced in multiparous cows by decreasing the change in starch content and fermentability from the dry diet to fresh diet. This study emphasizes the need to properly feed the fresh cow to maintain integrity of the rumen epithelium and support rumen health.

In addition to diet composition and the concept of a smooth nutrient transition from the dry period to the fresh period, feeding management in the fresh pen is important to minimize the risk of SARA and excessive inflammation (Zebeli et a., 2015). Large meals consumed quickly and infrequently (i.e. slug feeding) reduce salivary secretion and the buffering capacity of the rumen. Facilities and management practices (e.g. understocking the feed bunk) that promote smaller meals consumed more slowly and more frequently are preferred. Minimizing sorting of the diet is critical during the fresh period andcan be achieved by chopping dry forages to small size, adequately mixing the diet, feeding more frequently, and routinely pushing up the diet in the feed bunk.

▪ Conclusions

Early lactation diets, in particular fresh diets, should be formulated to maximize DMI and energy intake, prevent compromised lipid mobilization and SARA, and support a return to positive energy balance in order to optimize lactational and reproductive performance. There is no "one size fits all" fresh cow diet because the interaction of nutrition, environment, and management is unique for every dairy. However, use of a fresh cow group and diet for 10 to 21 DIM is recommended. The fresh diet should be formulated within the context of the dry and high diets. In general, following a low starch controlled or moderate energy dry diet, the fresh diet should not exceed ~25% starch or the amount that will be fed in the high diet, should avoid inclusion of higher fermentable starch sources, and should provide adequate peNDF to maximize DMI while minimizing SARA. The effectiveness of the fresh cow feeding program and management should be assessed by monitoring clinical and subclinical health problems, rumination, and variation in intake, milk yield, and body condition or weight loss.

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Relationship Between NDF Digestibility and Animal Performance

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▪ Take Home Messages

- ▸ An accurate laboratory measure of fibre digestibility is essential to optimize the utilization of forages in dairy cattle diets.

- ▸ The measure should mimic *in vivo* digestion and should be consistent across forage types.

- ▸ A new *in vitro* lab assay has been developed that predicts total tract NDF digestion (TTNDFD) in ruminants. The test is based on a patented and licensed *in vitro* assay and model of fibre digestion.

- ▸ The *in vitro* TTNDFD assay is available through commercial labs and has been calibrated to NIR analysis.

- ▸ The TTNDFD model predicts fibre digestion of alfalfa, corn silage, and grass forages in cattle and has been validated against directly measured NDF digestibility in lactating dairy cattle.

▪ Introduction

The digestibility of neutral detergent fibre (NDF) is more variable than the digestibility of any other feed component and can profoundly affect intake and milk production. In high producing dairy cows the variation in total tract fibre digestion can account for enough energy to support as much as 4 to 5 liters of potential milk yield. Fibre digestion is affected both by characteristics of the plant material and by the animal consuming the fibre. To accurately predict how fibre will be utilized, laboratory measures that predict the rate of fibre digestion and the proportion of total fibre that is potentially digestible are needed. The rate and potential extent of NDF digestion are heavily influenced by the genetics and growing environment of the forage. Fibre digestion is also

affected by the rate of passage of the potentially digestible fibre through the animal's rumen and hindgut and therefore prediction of fibre utilization must also account for animal.

▪ Predicting Fibre Digestion with Laboratory Tests and Modeling

There are at least 4 factors that affect fibre digestion and performance in ruminants:

  (a) the proportion of fibre that is potentially digestible

  (b) the rate of fibre digestion

  (c) the rate of passage of fibre in the animal

  (d) the proportion of fibre digestion occurring in the rumen and the hindgut (Figure 1).

The forage and the environment in which the forage was grown have the greatest influence on proportion of fibre that is digestible and the rate of fibre digestion. The animal eating the forage has the greatest influence on rate of passage and rumen/hindgut digestion. Each of these 4 parameters can be estimated with either lab tests or from existing research.
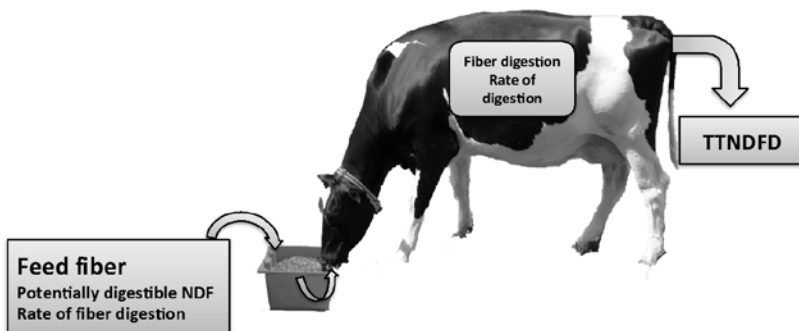
Figure 1. TTNDFD model

a. The proportion of feed fibre that is potentially digestible (pdNDF)

Fibre is bulky and one of the slowest digesting components of the diet, and clearance of fibre from the rumen is an important factor limiting feed intake and energy. Neutral detergent fibre consists of 2 components, potentially digestible (pdNDF) and indigestible NDF (iNDF). The proportion of NDF in the pdNDF fraction varies due to feed type and growing environment. On average, the pdNDF fraction of alfalfa is about 60 to 65% of total NDF. The proportion of potentially digestible fibre in corn silage is typically greater (75 to 85%) than in alfalfa NDF.

The iNDF fraction is estimated from long term incubations of fibre in the rumens of cattle or long term *in vitro* digestions. The NDF residue remaining after 240h of incubation ($uNDF_{240}$), is often used as an estimate of iNDF. The pdNDF is determined by subtracting the uNDF fraction from total NDF.

$$pdNDF = NDF - uNDF_{240}$$

The iNDF proportion can only be cleared from the digestive tract by passage whereas the pdNDF fraction disappears by passage and by microbial digestion.

b. The rate of digestion of potentially digestible fibre (kd)

The rate of fibre digestion also differs due to forage type and growing environment. The potentially digestible fibre in alfalfa is digested nearly twice as fast (4–6% per hour) as the potentially digestible NDF in corn silage (2–3% per hour). Even though fibre digestion rates for forages are slow, differences in rate of fibre digestion have a big impact on how much of the potentially digestible fibre will be digested. The total-tract NDF digestibility of alfalfa and corn silage is similar, but the process of NDF digestion is quite different. In corn silage, there is a larger fraction of digestible fibre that is digested slowly. In alfalfa, there is a smaller proportion of digestible fibre, but the faster rate of digestion of the potentially digestible fraction compensates for the bigger pool of iNDF. For the animal, the most important outcome is the total amount of fibre that is digested because digested NDF is a source of digestible energy.

c. The rate of passage of potentially digestible NDF through the cow (kp)

Both cow size and feed intake affect the passage rates of pdNDF and iNDF. Passage of fibre is much slower than the passage of forage dry matter. The passage rates of iNDF and pdNDF are also not the same. Passage of the

pdNDF fraction is slower than passage of the iNDF fraction (Lund et al., 2007).   As intake goes up, the rate of passage of both fractions also increases, and as a result, NDF digestibility declines.

d. Ruminal and hindgut fibre digestion

Approximately 90–95% of fibre digestion occurs in the rumen (Huhtanen et al., 2010), but digestion beyond the rumen must be accounted for if one is to accurately predict the amount of energy derived from NDF. When both ruminal and hindgut digestion are accounted for, a total tract NDF digestion (TTNDFD) measurement can be calculated and this digestion coefficient can be directly validated with dairy cattle.

An accurate assessment of fibre digestion requires that the 4 factors be integrated into a single measurement.

Total tract NDF digestibility = rumen digested NDF + hindgut digested NDF

The rumen fibre digestion process can be described mathematically as:

$$\text{Rumen digested NDF} = \text{pdNDF} \times ((kd)/ (kd + kp)$$

where pdNDF is the fraction or amount of potentially digestible NDF, kd is the rate of digestion of potentially digestible fibre from the rumen, and kp is the rate of passage of potentially digestible NDF from the rumen.

Hindgut digested NDF can be accounted for by dividing the NDF digested in the rumen by the proportion of total fibre digested in the rumen. In the TTNDFD model, it is assumed that 90% of total fibre digestion occurs in the rumen.

▪ Challenges with Assessing Forage Quality with $uNDF_{240}$, $NDFD_{30}$ or $NDFD_{48}$

Nutritionists use many different tests to assess fibre digestibility or to compare forages. The most commonly used assays are $uNDF_{240}$ or $NDFD_{30}$ or $NDFD_{48}$. The numerical subscripts indicate the time of incubation in rumen fluid. Assays that predict iNDF (such as $uNDF_{240}$), or *in vitro* digestion of fibre after a fixed time ($NDFD_{30}$ or $NDFD_{48}$) as stand-alone measures of forage quality have limitations. uNDF240 or NDFD values provide little insight into the energy content of the forage or its intake potential. These assays also cannot be used to compare across forage types or to formulate diets. These assays have value as a simple indexing tool, but they are not very accurate or precise stand-alone measures of forage quality.

Comparing forages with $uNDF_{240}$ ignores that rates of fibre digestion also vary within and between forages. A simple analogy demonstrates this point. Fibre quality is an estimate of the amount of digestible energy generated from a given quantity of forage NDF and is somewhat analogous to predicting how far you can drive a car before it runs out of fuel. You need to know how much fuel is in the tank and the fuel efficiency of the car to predict the distance that the car will travel. Forage quality is conceptually similar. The amount of digestible energy from fibre (how far you can drive a car) depends on the amount of fibre that is digestible (the amount of fuel) and the rate of fibre digestion (the fuel efficiency). A $uNDF_{240}$ value for forages is analogous to looking at the fuel gauge. Knowing you have half a tank of fuel (iNDF) in the car may be somewhat useful, but you can't accurately determine how far you can go unless you know the amount of fuel and the fuel efficiency of the car. If this particular vehicle had less than half a tank of fuel, it will not travel as far as if the tank was ¾ full, so looking at the gas gauge is a way to index the potential distance that could be traveled in this particular vehicle, but you don't know how many kilometers you can go. The iNDF values of 2 forages may be a tool to compare the relative values of forage fibre quality but it is not an accurate estimate of forage quality. Two different vehicles, each with half a tank of fuel will not necessarily travel the same distance because their fuel tanks may differ in size and the vehicles may differ in fuel efficiency. Knowing the proportions of indigestible NDF and pdNDF is somewhat useful but not a complete picture of fibre quality because both the amount of pdNDF and the rate of fibre digestion differ between different forages. The driver would also have some bearing on the distance traveled. If the driver has a 'lead foot' the distance traveled will be less than for someone who is a more conservative driver. This is a bit like the effect of rate of passage on fibre digestion. A high producing dairy cow is less efficient at digesting fibre because she eats more, which increases rate of passage. To calculate how far you can travel in a specific vehicle, you need to account for the amount of fuel (pdNDF), the fuel efficiency (kd) and the driver (kp). To quantify fibre quality you must integrate pdNDF, kd and kp into a single term.

The iNDF fractions and rates of fibre degradation can vary considerably within forage type. In forages measured in our lab, the iNDF fractions in alfalfa and grasses vary from less than 5% to over 55% of NDF, while corn silage iNDF values range from less than 10% to over 40% of NDF (unpublished data). Krizsan et al. (2010) reported that iNDF values in a database of 172 feeds ranged from 2.4 to 17.4% of feed dry matter. In addition, the estimated rates of degradation of pdNDF vary from about 1% per hour to over 10% per hour when measured by using multiple incubation time points and fitting the disappearance of pdNDF to first order kinetics. The TTNDFD *in vitro* assay is a more comprehensive measure of fibre quality than any of the individual terms that are used to determine fibre utilization.

In vitro NDF digestibility measured after 30 hours ($NDFD_{30}$) or 48 hours ($NDFD_{48}$) is widely used to index forage fibre digestibility. Oba and Allen (1999) reviewed several feeding studies with dairy cattle and concluded that a 1% change in *in vitro* or *in situ* NDF digestibility ($NDFD_{30}$ or $NDFD_{48}$) was correlated with a 0.17 kg increase in voluntary dry matter intake, and a 0.25 kg increase in 4% fat corrected milk yield. The change in *in situ* or *in vitro* fibre digestibility within a study was correlated with intake and milk production, but there was no significant correlation between the absolute measures of fibre digestion and intake or milk yield across studies. For field nutritionists, this suggests that *in vitro* methods differ enough from lab to lab to make it impractical to compare results between labs or to compare NDFD values of alfalfa to NDFD values of corn silage.

There is also another challenge with using values like $NDFD_{30}$ to assess forage quality. The NDF residue remaining after a given time in a flask of rumen fluid is simply undigested NDF. That residue consists of indigestible NDF and the portion of the potentially digestible NDF that has not yet been digested. There is no way of knowing or estimating the rate of fibre digestion or the fraction of indigestible NDF from this measurement alone. In addition, the *in vitro* and *in situ* analyses are closed systems, which means that rate of passage of fibre is not accounted for.

▪ *In vivo* Measurement of Fibre Digestion

Total tract apparent NDF digestibility values for diets fed to dairy cows are readily available and are a valuable tool for field nutritionists. Goeser (2008) summarized total tract NDF digestibility measurements that were reported in 25 corn silage feeding trials (81 treatment comparisons) and in 20 trials in which legumes and grasses (64 treatment comparisons) were the primary forages fed to high producing ruminants. Summary statistics suggest that *in vivo* NDF digestibility coefficients can vary by 30 to 35% units among legumes, grasses and corn silages. The TTNDFD of corn silage based diets average about 42% of NDF but range from 20% to nearly 60% of NDF. Diets for high producing dairy cows are typically formulated to contain between 28 and 35% total NDF. For cows that are expected to produce over 45 liters per day, a 30-unit change in total tract NDF digestibility with diets that contain similar amounts of NDF is equivalent to the digestible energy needed to support more than 4.5 liters of milk production.

Measuring the Fibre Digestion Process *in vivo* with the Rumen Evacuation Method

Measuring the process of ruminal and hindgut fibre digestion *in vivo* is laborious and expensive, but is the 'gold standard' to which other estimates of fibre digestion should be compared. Comprehensive evaluations of *in vivo*

fibre digestion are most commonly measured by the 'rumen evacuation' technique. With this method, the critical dynamic components that contribute to the digestion of fibre are directly measured in rumen-cannulated animals. Rumen pools of digestible and indigestible fibre are measured by total rumen evacuation. Rates of digestion of potentially digestible NDF and rates of passage of pdNDF and indigestible NDF are also measured as well as total tract NDF digestion.

Despite the cost and labor, a large number of rumen evacuation studies have been published from studies done in the US and Northern Europe with dairy cattle. Krizsan et al. (2010) compared ruminal passage rates of indigestible NDF as measured by the rumen evacuation technique to empirical estimates of particulate passage rate in cattle. Their database included 49 studies in which 172 treatment means were measured. From this database, they published predictive equations for passage of iNDF in lactating cow fed diets based on corn silage, grass silage, alfalfa and pasture-based grass diets. Huhtanen et al. (2010) also published a meta-analysis of the NDF digestion process using the rumen evacuation method 32 studies and 122 diets were included in this analysis. Most of the published studies are with lactating dairy cows fed grass, alfalfa or corn silage based diets. The fibre digestion module of the recently published Nordic Feed Evaluation system (NorFor) is based on fibre kinetic parameters estimated by the rumen evacuation technique (NorFor, 2011).

The rates of pdNDF degradation of diets when measured by the rumen evacuation method typically range from approximately 2% to 6% per hour. Corn silage based diets typically have slower rates of pdNDF degradation than alfalfa. The NDF in diets based on temperate grasses tends to have a similar proportion of pdNDF as corn silage, but grass fibre degrades faster than corn silage fibre, but slower than alfalfa fibre.

Predicting *in vivo* NDF Digestion with the University of Wisconsin *in vitro* TTNDFD Assay

University of Wisconsin researchers have recently developed an *in vitro* lab assay and model for predicting NDF digestion in dairy cattle that can be used by field nutritionists. The outcome is a total tract digestibility coefficient for NDF (TTNDFD). The TTNDFD value is benchmarked to fibre digestibility values that have been obtained from feeding studies where NDF digestion has been directly measured. Total tract fibre digestibility is reported because this value can be used not only to predict *in vivo* fibre utilization but also to predict forage DE, NE or TDN values.

The TTNDFD assay accounts for pdNDF, kd, kp and hindgut digestion of NDF (Figure 1). Measurement of the pdNDF fraction and the kd of pdNDF are

based on a modified Goering and Van Soest (1970) *in vitro* procedure (Goeser and Combs, 2009). The pdNDF fraction is estimated from long term (120 or 240 h) *in vitro* incubations. Multiple measurements of *in vitro* NDF digestibility are used to calculate a rate of ruminal pdNDF digestion. The approach accounts for ruminal and post-ruminal fibre digestion and can be adjusted for changes in fibre passage as size or intake of the animal changes. Rates of fibre passage are estimated from regressions that have been derived from *in vivo* studies (Krizsan et al., 2010, Lund et al., 2007). In this model, the diet TTNDFD can be calculated by summing the amount of digestible fibre provided from each feed. The *in vitro* method has been calibrated to Near Infrared Spectroscopy (NIR) so that kd and iNDF fractions in a feed can be predicted quickly and with little additional cost.

Several feeding studies have been conducted with various forages to test the model and to validate that the estimates of digestion and passage that are used in the model are consistent with what is measured in cattle fed diets containing the test forages (Lopes et al., 2015a,b,c). In addition, our lab group has been monitoring commercial lab-derived TTNDFD for corn silages, alfalfa and grass forages and comparing these values to the digestibility coefficients for the respective forages that have been published in peer-reviewed feeding studies.

Field Observations with TTNDFD

We have been monitoring the TTNDFD values of corn silages, alfalfa and grasses that have been submitted to a commercial forage-testing lab for routine analysis. The TTNDFD values for corn silage, alfalfa and grasses are summarized in Table 1. The average values represent over 7000 samples each of corn silage or alfalfa and over 1200 grass forage samples.

Table 1. Typical TTNDFD values of corn silage, alfalfa or grass*.

	TTNDFD, % of NDF	SD	Range
Corn silage	42	± 6	20-60
Alfalfa	42	± 7	25-80
Grass	47	± 8	6-80

*Samples submitted to Rock River Laboratories, Watertown, WI.

The means, standard deviations (SD) and ranges in TTNDFD values coincide with *in vivo* measures of total tract NDF digestibility that have been reported in dozens of controlled feeding studies published in peer reviewed journals. For consultants, we recommend that tested forages be compared with these mean TTNDFD values. When comparing 2 forages with similar total NDF, a forage that is more than 1 SD below the mean TTNDFD value would be

among the lowest 15% of forages sampled and a 6 to 7 unit difference from the mean TTNDFD value would indicate that his forage fibre would reduce the DE value of the forage by enough to reduce potential milk yield by 1 to 1.5 liters. A forage which is 1 SD above the mean TTNDFD value would be higher in fibre digestibility than 85% of the forages tested and would contain enough additional DE to potentially support 1 to 1.5 liters more milk production. Experiences with this test in the field suggest that diets that incorporate large amounts of low TTNDFD forage support less milk and cows consume less feed dry matter than expected. Cows fed these types of diets respond well to additions of extra starch, or addition of sources of more highly digestible fibre, such as soy hulls.

Validation with Controlled Feeding Studies

The laboratory prediction of TTNDFD of forages and diets has been validated to fibre digestibility values that have been directly measured in feeding studies. One study (Lopes et al., 2015a) was designed to compare estimates of ruminal fibre digestion predicted from *in vitro* NDFD analysis of feeds to the ruminal fibre digestion measured in cattle fed the same feeds. The feeding study was conducted with lactating dairy cows fed either low fibre digestibility corn silage or to higher fibre digestibility corn silage as the main source of dietary NDF (Table 2).

Table 2. Effects of source of corn silage on total tract NDF digestion (Lopes et al., 2015a)

Feed, % of TMR DM	LFDSCS[1]	HFDCS[2]	SE
Low fibre digestibility corn silage	47	0	
High fibre digestibility corn silage	0	47	
Alfalfa silage	17	13	
Concentrate mix	36	40	
Diet composition			
NDF, % of DM	27.5	28.3	
pdNDF, % of NDF	68.9	75.9	
Results			
DMI, kg/d	25.5	25.6	1.3
4% FCM, kg/d	34.3	34.9	1.0
Observed TTNDFD (in vivo), % of NDF	47	49	2.5
Predicted TTNDFD (in vitro), % of NDF	43	50	0.9

[1,2]Low fibre digestibility and high fibre digestibility corn silage, respectively.

The fibre characteristics of the low fibre digestibility corn silage (34.4% NDF, pdNDF 58.6% of NDF, kd 3.2%/h) and the higher fibre digestibility corn silage (38.4% NDF, pdNDF 74.3% of NDF, kd 3.3%/h) were determined by our *in vitro* TTNDFD method prior to the feeding experiment. The fibre

characteristics of the 2 silages and the other feeds used in the diets were then used to predict total tract NDF digestibility of the treatment rations. The predictions for each diet were then compared to the observed measures of fibre digestion in dairy cows fed the same feeds. The *in vitro* method predicted that the higher fibre digestibility corn silage was higher in TTNDFD than the low fibre digestibility corn silage because it contained a larger proportion of potentially digestible NDF. Rates of pdNDF digestion and passage and the pool of pdNDF in the rumens of cows fed the experimental diets were directly measured in cows and compared to the fibre digestion parameters from the TTNDFD assay and model. It is important to note that the fibre digestion parameters measured directly in the cows are independent of the *in vitro* measurements. Results of the study indicate that the *in vitro* TTNDFD were similar to the directly measured *in vivo* total tract NDF digestibility values and provide evidence that supports the concept that *in vivo* fibre digestion can be predicted from *in vitro* fibre kinetics.

The objective of another *in vivo* experiment (Lopes et al., 2015b) was to compare estimates of total tract fibre digestion as predicted by the *in vitro* TTNDFD model to *in vivo* measurements in lactating dairy cows. Cows were fed diets that varied in proportions of corn silage and alfalfa. The *in vitro* fibre digestion parameters for corn silage (NDF = 34.4%, pdNDF kd = 3.2%/h, pdNDF = 58.6% of NDF) and alfalfa silage (NDF = 34.7%, pdNDF kd = 6.1%/h and pdNDF = 51.3% of NDF) indicate that fibre in the corn silage contains more pdNDF than alfalfa, but the rate of digestion of alfalfa fibre is nearly twice as fast as corn silage fibre. The feeding experiment measured how cows use forages that differ in pdNDF and kd (Table 3). The diets contained approximately 55% forage and the dietary NDF concentration was similar across the 4 treatments.

Table 3. Comparison of rumen and total tract NDF digestion of diets predicted from TTNDFD model and observed *in vivo* (Lopes et al. 2015b)

Item	Predicted *in vitro*[1]	Observed *in vivo*[2]	SEM	P value
Input				
pdNDF kd, %/h	4.1	4.3	0.5	0.72
pdNDF kp, %/h	2.7	2.8	0.3	0.56
Output				
NDF digested in rumen, kg	2.73	2.63	0.22	0.64
NDF digested in hindgut, kg	0.36	0.64	0.19	0.05
NDF digested in total tract, kg	3.09	3.27	0.22	0.42
Total tract NDF digestibility, % of NDF	46.4	49.5	0.07	0.13

[1] Fibre digestion parameters predicted from *in vitro* analysis of feed components of the diets before cows were fed test diets.
[2] Fibre digestion parameters directly measured in cows fed the test diets.

Feed intake was lower when cows consumed the diets that contained 100% of forage as alfalfa silage than it was when cows were fed diets containing

corn silage. The observed (*in vivo*) total tract NDF digestion values were calculated from feed and fecal samples. Cows consuming the diet with alfalfa as the only forage had higher NDF digestibility than cows on the diets that contained corn silage. Milk and fat corrected milk yield did not differ due to treatment. The NDF digestibility coefficients predicted by the *in vitro* TTNDFD method were similar to the *in vivo* values. The fibre digestibility coefficients suggest that the faster rate of fibre digestion of alfalfa fibre compensates for lower content of pdNDF but as higher proportions of alfalfa forage are fed, the amount of indigestible fibre in the rumen increases and rumen fill becomes a more predominant factor limiting feed intake.

These feeding experiments demonstrate that the *in vitro* TTNDFD analysis can provide important insights into fibre utilization by dairy cattle. The rates of fibre degradation determined from the *in vitro* NDFD assays are consistent with values measured in *in vivo* feeding studies. The kd, kp and pdNDF parameters predicted by the TTNDFD model appear to be consistent with *in vivo* measures and the total tract digestion of NDF as predicted by the TTNDFD model is consistent with observed *in vivo* digestion.

The third study (Lopes et al., 2015c) compared 21 diets from 7 feeding experiments and showed that TTNDFD of total mixed rations analyzed by the *in vitro* TTNDFD method were highly correlated to the directly measured in vivo total tract NDF digestibilities of the same diets in lactating dairy cows.
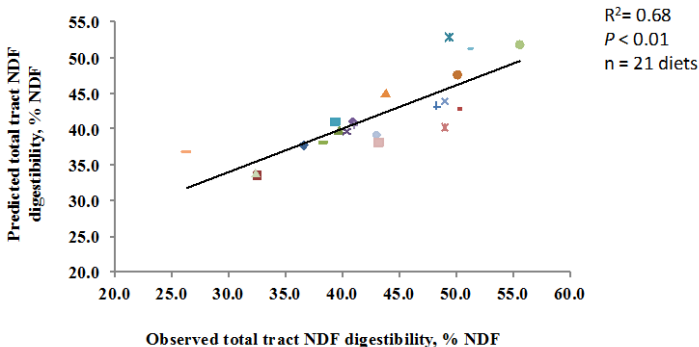

Figure 2. Comparison of observed total tract NDF digestion and NDF predicted by an in vitro TTNDFD assay 21 diets from seven feeding studies. Lopes et al., 2015c.

▪ Conclusions: How to Use the TTNDFD Test

The key to getting the most out of forages is understanding how energy values are affected by NDF and NDF digestibility. This test is intended to be an additional tool to provide a clearer understanding of how forage-fibre is utilized in dairy cattle. It is not intended to be the only tool to be used to evaluate forage quality or fibre utilization by dairy cattle. Table 5 summarizes important limitations to this assay. In top quality forages, NDF accounts for 35-45% of the total dry matter and this fibre is the source of 30 to 40% of the digestible energy. A 30% NDF diet with a TTNDFD of 33% would support 3 to 4.5 liters less milk than a 30% NDF diet with a TTNDFD of 45% assuming no reduction in feed intake. The average TTNDFD value for most diets formulated with alfalfa and corn silage will be about 42 to 44% and this should be a target for ration formulations.

Table 5. Guidelines for using TTNDFD.

1. The TTNDFD assay is intended to evaluate the digestibility of NDF of feeds and rations in animals fed a balanced diet. Inadequacies of other nutrients (protein, amino acids, minerals) or excesses of dietary components other than fibre (e.g., mycotoxins) are not accounted for in this assay.

2. The TTNDFD can be used to compare fibre utilization across forage or fibre sources. For example, fibre digestibility of corn silage can be compared to fibre digestibility of alfalfa, grass or co-product feed.

3. TTNDFD does not account for differences in physical form (effective fibre) of forages.

4. TTNDFD estimates total tract digestibility of fibre for a dairy cow consuming about 24.5 kg of DM.

5. *In vitro* NDFD values (NDFD24, NDFD30 or NDFD48) should not be used as a single indicator to compare fibre digestibility of forages. These values do not factor in indigestible fibre, or NDF concentration of forages. Single time NDFD values are poorly correlated to total tract fibre digestibility

6. Total NDF and TTNDFD must be considered when comparing forages for quality.

The TTNDFD value can also be used as a stand-alone value to index forages. A consultant could compare values from their forage test to the values in

Table 1. For example, note in the Table 1 that an average alfalfa will have a TTNDFD value of 42%. An alfalfa with a TTNDFD value one standard deviation below average (less than 36%), would be among the bottom 15% of the alfalfas tested. A sample with low TTNDFD likely will not be utilized as well as 'typical' alfalfa containing similar amounts of total NDF. Our validation studies with corn silages, alfalfa and temperate grasses indicate that TTNDFD values of feeds can be used in ration formulation and evaluation to 'fine-tune' the amount and overall digestibility of NDF in rations of high producing dairy cattle. The ability to predict fibre digestibility and incorporate this information into rations could improve our ability to optimize forage utilization and milk production.

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Dairy Processor's Role in Promoting Animal Welfare

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▪ Take Home Messages

▸ As an important stakeholder in the dairy value chain, dairy processors have a fundamental interest in animal welfare assurance.

▸ The processing industry has become fully engaged in ongoing development of animal care Codes of Practice/Standards and the assessment programs that provide compliance assurance.

▸ Recent high profile cruelty incidents in Canada and the USA have been 'game-changers' for the industry as a whole and in particular for dairy processors. Despite the negative media attention, such events do bring positive change, especially in Canada:

  o Stronger commitment to Code compliance and animal care assessment to mitigate risk of cruelty incidents from occurring.

  o Recognition of the need to develop policy and protocols to address cruelty incidents in order to maintain customer and consumer confidence.

▸ Key welfare issues on the Canadian horizon from the processor's viewpoint:

  o Animal care assessment – need to have a robust program that provides assurance to meet industry, customer and consumer expectations.

  o Industry needs to address some specific welfare challenges:

    ▪ Elimination of tail docking.

    ▪ Pain control for routine management procedures.

    ▪ Animal handling training, Codes of Conduct.

    ▪ End of life decision-making, cull cow transport.

▪ Saputo's Heightened Involvement in Animal Welfare

Saputo is a Canadian-based dairy processing company founded by an Italian immigrant family in post WWII Montreal in the 1950's. It has grown to a multi-national company with operations in Canada, USA, Australia and Argentina. The company has >12,000 employees in 55 plants and is one of the top processors in the world and the largest in Canada.

Like most animal product food processing companies, Saputo has an animal welfare policy posted on its corporate website. The original policy was quite general, stating that the 'company shares industry and society's concerns about animal welfare', and that Saputo 'appreciates animal welfare standards and practices are required, and expect our suppliers to adopt proper animal care methods.'

In June of 2014, an animal cruelty incident occurred on a large dairy farm in Chilliwack, British Columbia. The animal rights activist group Mercy for Animals revealed undercover video evidence of farm employees abusing dairy cows (beaten, kicked, dragged, etc.) at the entrance to the rotary milking parlour. An animal protection enforcement investigation was conducted and criminal charges were recommended. This case brought on a torrent of negative traditional and social media outcry. Saputo was targeted by activists suggesting this incident occurred on a "Saputo farm" and that the company has control of on-farm animal welfare. A Change.org petition was started demanding that Saputo "*stop supporting horrific animal abuse*", and urged consumers to stop buying Saputo products until "*it does the right thing*". As has happened in other similar cruelty incidents, major multinational companies and their brands will be targeted also. This results in loss of market as customers cannot accept products made from milk where such incidents have occurred until producer reintegration steps have been taken. This was a 'game-changing' moment for our company and for the processing industry. It was clear that Saputo would need to develop a proactive policy that focused on a protocol to deal with cruelty incidents and also with compliance with animal care Codes of Practice/Standards.

▪ Saputo's Animal Welfare Policy

Saputo has developed a new global animal welfare policy appropriate for all jurisdictions (the company has operations in Can, USA, Aus and Arg). The goal was to have a policy that is progressive and shows industry leadership in dairy cattle welfare. It was launched in June 2015 (Saputo Animal Welfare Policy, 2015).

The policy is based on the following principles:

‣ Animal welfare is a 'pre-competitive' issue – similar to food safety.

‣ Policy is science-based and aligned with recognized national care and handling Codes/Standards and assessment programs.

The policy has two key elements:

‣ **Zero tolerance for any act of cruelty**: When Saputo is presented with credible evidence to support an allegation of animal cruelty, milk receiving is suspended until an animal protection enforcement investigation is conducted and an independent veterinary welfare audit has been performed.

‣ **Compliance with recognized national Codes/Standards for proper animal care and handling**: In Canada, this is the National Farm Animal Care Council (NFACC) *Code of Practice for the Care and Handling of Dairy Cattle* (NFACC, 2009). In the USA, it is the National Milk Producers Federation *Farmers Assuring Responsible Management (FARM)* program (National Milk Producers Federation). Compliance must include appropriate animal handling training and an animal care agreement (Code of Conduct).

▪ Processor's View of Key Welfare Issues on the Horizon

I. **Animal Care Assessment**: Processors are looking forward to the full implementation of the animal care module of proAction® to provide assurance of compliance with Codes/Standards (DFC, 2015). A robust assessment program is key to meeting industry expectations and maintaining trust in the dairy sector. Such programs must mature to a format that incorporates validation by a third party audit/verification to meet the expectations of industry, customers and consumers.

Veterinarians will play a key role in helping producers understand Code compliance, prepare for assessments and follow-up on any corrective actions necessary to maintain compliance (e.g. lameness identification/treatment/prevention, euthanasia protocols, cull cow transport decision-making).

II. **Addressing Specific Industry Welfare Challenges**: Our industry needs to commit to addressing some specific management practices and animal care/handling issues that present ongoing challenges to welfare and risk eroding consumer and social trust.

a. Tail docking – The practice of tail docking cattle must be eliminated. It has been performed based on the assumption that it will decrease the risk of udder infections, contribute to cleaner cows and improve the working conditions of those handling dairy cows. Scientific evidence has not identified any difference in udder or leg hygiene, somatic cell count or prevalence of intrammamary infections (Tucker et al., 2001). Welfare concerns include pain or discomfort, risk of neuroma formation and post-operative infections and loss of ability to control flies (Eicher et al., 2006). The Canadian, American and Australian veterinary medical associations all oppose the routine tail docking of cattle.

b. Pain control for disbudding/dehorning – The use of pain control (appropriate anesthesia and analgesia) when disbudding or dehorning cattle must become a minimum industry standard. The prevention of horn growth by genetic selection and breeding or polled stock is achievable, but polled dairy sire selection is currently very limited. Where genetic selection for polled stock is not an option, calves should be disbudded in preference to dehorning using anesthesia and post-operative analgesia (Stafford and Mellor, 2005; Stewart et al., 2009). The Canadian, American and Australian veterinary medical associations all recommend the use of pain control for disbudding and dehorning of dairy cattle.

c. Animal handling training – All those that handle dairy cattle should be appropriately trained in quiet cattle handling methods using a recognized training program. Quiet handling methods reduce fear, avoid injury, make observation and treatment easier and enhance animal well-being and productivity. Animal handling training must include education on non-ambulatory cow care and proper use of electric prods. Prods should only be used in extreme situations (never on sensitive areas, e.g. face, udder, genitalia).

Every person who handles or comes into contact with an animal should sign a cow care agreement (Code of Conduct). Such agreements provide everyone on the farm with a clear understanding of farm policies and highlight the importance of appropriate animal care. They provide an understanding of:

   i. farm owner and employees' commitment to doing the right thing, and outlines what must happen when things go wrong

   ii. protocol that must be followed if any person witnesses an act of animal abuse, mistreatment or mishandling

d. End of life decision-making, cull cow transport – The dairy industry is not often associated with the slaughter of animals, and as a result cull cow welfare, until recently, has remained out of the

spotlight. There are ongoing concerns with poor decision-making resulting in the transport of unfit cull dairy cows and veal calves to auction markets (e.g. severe lameness, emaciation, weak/dehydrated calves). Many such animals are either euthanized or sent back to the farm of origin. The dairy industry needs to address this welfare concern by educating all involved in the marketing chain (producers, drovers, veterinarians, auction market owners/employees):

    i. Producers must understand the complex marketing pathways that cull cows often face. The cows spend an average of 7-9 days in transit until they are slaughtered. Many producers believe when they ship a cow for beef they are slaughtered the next day!

    ii. There needs to be education/training on recognizing animals that are compromised (e.g. cow with LDA + dehydration) and unfit for transport (e.g. severe lameness).

    iii. Producers need help developing SOPs for cull cow and calf transport using established decision trees. Market chain stakeholders need to understand and implement alternative marketing options for compromised cull cows that require special handling (e.g. local slaughter, 'direct to slaughter' at auction market, on-farm slaughter).

The dairy processors are engaged with other stakeholders in the dairy value chain as we all move forward on the path of advancing animal welfare. We must all be working toward robust animal welfare assurance provided through an animal care assessment program that validates compliance with the Code of Practice in order to maintain customer and consumer confidence and trust.

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Promoting Animal Welfare – An Industry Perspective

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▪ Take Home Messages

▸ Animal care is essential on every dairy farm and is an integral part of daily life for every dairy farmer.

▸ Canadian dairy farmers have collectively agreed to develop and implement a national mandatory animal care program to demonstrate to consumers and customers how they care for their cattle.

▸ The Animal Care program is based on the *Code of Practice for the Care and Handling of Dairy Cattle*, and is part of Dairy Farmers of Canada's proAction® Initiative.

▪ Introduction

Consumers and customers are increasingly demanding assurance that the food they are buying is safe, wholesome and produced responsibly. Today, their demands have moved beyond the safety and quality of the product itself, to wanting evidence that the farmer, processor, retailer and/or food company can demonstrate in a credible way that they have valid and sustainable practices when they produce food.

Canadian dairy farmers have decided to show their sustainable practices and the progress they make on farms by developing nationally the proAction® Initiative. Building on the Canadian Quality Milk program, which farmers have implemented to show they address food safety risks on farms, proAction is being developed by farmers for farmers, with various experts involved, and is designed to be practical, effective and credible.

Animal Care and Livestock Traceability are the next priority areas of proAction to be implemented, with on-farm training having started in the fall of 2015. Every farmer knows that excellent cattle care is the foundation of a successful

dairy farm. Farmers strive to continuously improve the practices on their farms and they spend many hours every day with their animals: milking, feeding, and monitoring their health and wellness. With proAction, farmers will demonstrate and measure their commitment to best management practices and continuous improvement.

▪ ProAction Initiative

Dairy Farmers of Canada's proAction Initiative provides assurance to customers about farm practices, integrating six modules under one umbrella:
- o Milk Quality
- o Food Safety (Canadian Quality Milk program)
- o Animal Care
- o Livestock Traceability
- o Biosecurity
- o Environment

Canadian dairy farmers' vision for proAction is to collectively demonstrate responsible stewardship of their animals and the environment, while sustainably producing high-quality, safe and nutritious food for consumers.

Each module of proAction is being developed and implemented in a staged approach so that farmers have adequate time to learn about the requirements and implement them on their farms.

The final program materials for Animal Care and Livestock Traceability were published on September 1, 2015, in time to initiate the training and communication phase to farmers and stakeholders. Farmers have two years to learn about the programs, train staff and adjust their practices, as necessary, to meet the programs' expectations.

In September 2017, the Animal Care and Traceability requirements will be incorporated into the validation process. From September 2017 on, when farms are due for a Food Safety validation, their implementation of the Animal Care and Livestock Traceability requirements will be evaluated as well.

An on-farm pilot to test the draft requirements of the Biosecurity and Environment modules was started in early 2016. Dairy Farmers of Canada (DFC) is planning to start the training phase of the Biosecurity program in September 2017, and then incorporate Biosecurity into the validation process in September 2019.

The Environment will follow two years later, with training for farmers starting in September 2019, and then incorporation into the validation process in September 2021.

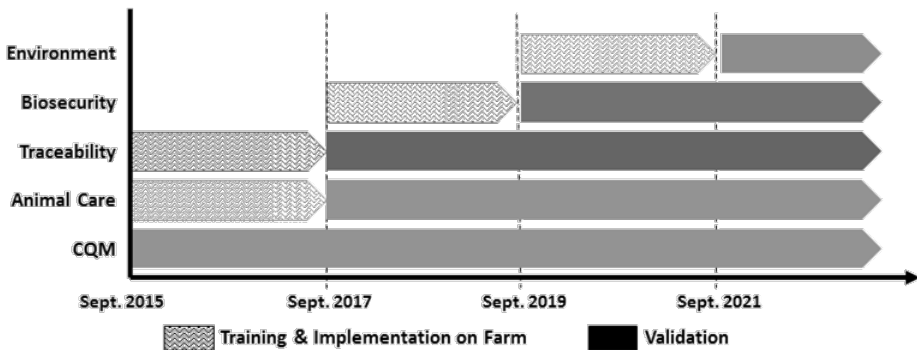Figure 1 illustrates the timelines associated with the phases of each program.

Figure 1: Development and implementation timelines for proAction

▪ Animal Care Program

Background

Canadian dairy farmers have been investing in research to support the development of science-based practices to continuously improve the care of dairy cattle for almost two decades. In 2009, the National Farm Animal Care Council (NFACC) and DFC published "*The Code of Practice for the Care and Handling of Dairy Cattle*" (hereby referred to as the "Code of Practice"). The Code of Practice is a national guideline that outlines best management practices and requirements for dairy farmers related to animal care. The Code of Practice is science-based.

In 2010, DFC co-financed a major research project under the Dairy Research Cluster that was designed to measure and benchmark animal comfort on Canadian dairy farms and develop standard operating procedures (SOPs) to help farmers measure the status of their cows' comfort.[1]

Using the outcomes of this research, DFC participated in a NFACC project to pilot the Animal Care Assessment Framework, which is a process that outlines how to translate the requirements of a Code of Practice into an auditable, on-farm, animal care program. The project involved a small pilot on dairy farms to test the program requirements and their practical application.

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[1] Animal Comfort Tool, https://www.dairyresearch.ca/animal-comfort-tool.php

DFC then revised the program according to the feedback and launched a second, larger pilot on dairy farms across the country. Farmers provided excellent feedback, and commented that the program helped them identify which areas they were addressing well and which areas needed greater focus. Once this pilot was completed, DFC finalized the program requirements, and presented the final program to its General Council during the DFC Annual General Meeting in July 2015. The General Council approved the program.

Requirements

The Animal Care program is based on the requirements outlined in the Code of Practice, and, as such, it is designed to assess a dairy farm's level of compliance to the Code of Practice. The main areas the Animal Care program addresses are: dairy facilities (e.g. housing design, bedding, and space), feed and water, animal health, handling and shipping animals, and staff training and communication.

The Animal Care program contains animal-based measures to assess the welfare of dairy cattle: records, such as SOPs, to demonstrate that certain procedures are documented and followed consistently by staff, and other best management practices such as housing design and cattle handling.

The animal-based measures are a key component of the program with solid foundations in scientific research financed by Canadian dairy farmers. Cattle are assessed for body condition score, injuries and lameness. These measurements are indicators of facility design, barn management, feeding practices, and health status of the individual animals. The initial assessment will be used as a benchmark for the farm, so that the farmer can identify opportunities for improvement. The assessment report will also show the farm how its results compare to other farms. Future assessments will provide farmers with the ability to measure and track their herds' status over time, and strive for continuous improvement.

Implementation

DFC launched the training phase of the Animal Care program and Livestock Traceability on September 1, 2015. DFC distributed materials to provincial organizations to enable them to implement their training plans with farmers. Provincial organizations will deliver the program directly to farmers, as they do with the Food Safety program.

The program materials are available on the proAction website: www.dairyfarmers.ca/proAction (click on "Resources" and then click on the Animal Care icon to find the Farmer Manual). Provincial associations

are offering workshops or other forms of training to farmers to learn about the program and to learn how to implement it on their farms.

In September 2017, DFC will launch the validation phase of the program, and the requirements will be integrated in the Food Safety program, and become mandatory elements for continued registration.

▪ Conclusion

Animal care is an essential element on every dairy farm. The Animal Care element of proAction is based on the requirements outlined in the *Code of Practice for the Care and Handling of Dairy Cattle*. DFC's goals with the Animal Care program are to provide recognition to those farmers who are doing an excellent job of caring for their dairy cattle, and to encourage farmers to strive for continuous improvement in animal care.

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The Role of Glucose in Dairy Cattle Reproduction

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▪ Take Home Messages

‣ Glucose is an important nutrient for the dairy cow because there is a high demand for milk production and it must be synthesized de novo in the liver.

‣ Glucose controls circulating concentrations of nonesterified fatty acids (NEFA) and beta-hydroxybutyrate (BHBA) in part through its effects on blood insulin concentrations.

‣ Improved immune function and shorter interval to first ovulation are 2 potential benefits to increasing circulating blood glucose, insulin, and insulin-like growth factor–1 (IGF1) and reducing NEFA and BHBA.

‣ Treating ketotic cows with propylene glycol (and thus providing substrate to increase blood glucose and lower blood ketones) improves their postpartum reproduction.

‣ Optimizing all aspects of herd nutrition beginning with the dry period is the best way to maintain adequate glucose supply so that postpartum reproduction is not compromised.

▪ Introduction

Glucose is a critical nutrient in the postpartum cow because it is a major component of cow's milk and also is a coordinator of the endocrine mechanisms controlling homeorhesis (Lucy et al., 2014). The sum of the affected mechanisms can impinge upon the cow's immune system, perhaps affecting postpartum health by affecting immune cells that combat common postpartum diseases such as metritis, endometritis, mastitis, and pneumonia (Moyes, 2015). The endocrine axes controlling the ovary are also affected to potentially influence the return to normal cyclicity (Lucy, 2008). This paper will

specifically focus on glucose because of the requirement for de novo synthesis in liver combined with its high demand in early lactation.

▪ Glucose in the Postpartum Cow

General Aspects of Glucose Metabolism

The microorganisms in the rumen ferment carbohydrates to volatile fatty acids (VFA) that can be oxidized for energy. In addition to VFA, protein and fat passing into the lower digestive tract are absorbed and used for the synthesis of milk protein and fat. Seventy-two grams of glucose are required for each kg of milk produced (Bell, 1995). Most of this glucose is converted directly into lactose (milk sugar). Glucose is rapidly fermented to VFA in the rumen and gastrointestinal tract and these VFA enter the circulation of the cow. Glucose is then resynthesized in liver from VFA as well as amino acids and glycerol by using a process called gluconeogenesis (Figure 1).
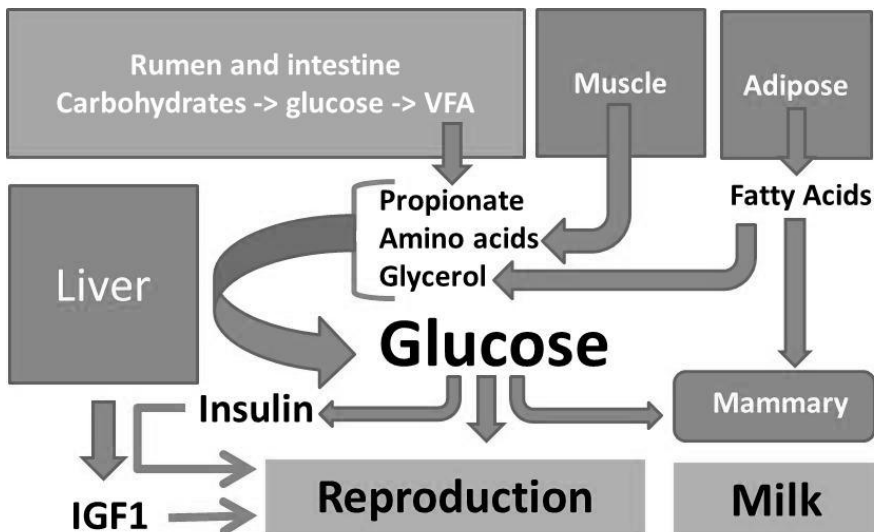

Figure 1. Metabolic processes in the early postpartum cow with potential to link glucose to the reproductive system (Lucy et al., 2014). Glucose is synthesized in the liver via gluconeogenesis from substrates arising from rumen fermentation and the catabolism of muscle and adipose tissue. Glucose may ultimately control both circulating insulin (directly) and liver IGF1 production (via insulin-stimulated IGF1 synthesis and secretion). Glucose is also a required substrate for lactose synthesis during the production of milk. Low circulating glucose may impair reproductive processes that are needed to re-establish pregnancy during early lactation.

Glucose Demand and Associated Homeorhetic Mechanisms

An early lactation cow will produce 50 to 100 kg of milk per day. This equates to a glucose requirement for milk production alone of 3.6 to 7.2 kg per day. The cow undergoes a series of homeorhetic mechanisms that are aimed toward elevating glucose supply (Bauman and Currie, 1980). In addition to a large increase in hepatic gluconeogenesis shortly after calving, the cow assumes a state of insulin resistance that prevents glucose storage as glycogen in muscle or liver, or to use glucose for lipogenesis in adipose tissue. The insulin resistant state conserves glucose for the synthesis of lactose in the mammary gland. In spite of the increase in gluconeogenesis and the development of insulin resistance, the postpartum cow has chronically low blood glucose concentrations because she fails to meet the glucose requirement for lactation.

Glucose as a Regulator of Postpartum NEFA and BHBA

Cows will break down glycogen in liver and muscle to release glucose early postpartum. The glycogen stores are quickly depleted. Cows also break down triglycerides in adipose tissue to yield glycerol (a substrate for glucose synthesis) and NEFA that can be used for energy. Typically, an excess of ketones are formed leading to elevated BHBA in blood. Increasing the circulating concentration of glucose by increasing glucose supply or decreasing demand rapidly decreases circulating NEFA and BHBA. This is because glucose can cause the release of insulin which will antagonize lipolysis and promote lipogenesis. Glucose also provides substrate to the tricarboxylic acid cycle so that BHBA can be fully metabolized (White, 2015). Thus, circulating glucose is an important regulator of both NEFA and BHBA.

Glucose as a Regulator of Postpartum Endocrine Function

In addition to its effects on metabolites, glucose can orchestrate changes in endocrine hormones such as insulin and IGF1 (Lucy, 2008). Glucose causes insulin release, and insulin partitions glucose toward adipose tissue and muscle by causing glucose transporters to move to the cell surface. Insulin also stimulates the liver to increase the expression of growth hormone receptors and release IGF1 into the circulation. As long as glucose remains low, insulin and IGF1 remain low, and the cow remains in a catabolic (tissue-losing) state during lactation. When the glucose supply increases (generally through greater gluconeogenic capacity) or the mammary gland produces less milk (gradually throughout lactation), then blood insulin increases. The increase in insulin causes the cow to partition glucose toward adipose tissue and muscle (an anabolic state). The switch from the catabolic state (low glucose, low insulin, and low IGF1) to the anabolic state (high glucose, high

insulin, and high IGF1) is a key regulator of the reproductive axis (Kawashima et al*.,* 2012).

▪ **Association Between Early Postpartum Glucose and Fertility Later Postpartum**

The blood concentrations of glucose decrease after calving. The decrease in blood glucose is theoretically caused by the rapid and sustained increase in glucose demand for milk production. Cows that become pregnant after first insemination have greater blood glucose concentrations on the day of calving and during the first 3 weeks after calving compared with cows that do not become pregnant (Garverick et al*.,* 2013). The relationship between blood glucose around the time of calving and improved reproduction is seen for cows in confinement (Garverick et al., 2013) and also for cows in pasture systems (Moore et al., 2014).

Mechanisms that determine the circulating concentration of blood glucose at or near the time of calving are not very well understood. Circulating blood glucose concentration is determined by entry rate, exit rate and pool size. Exit rate is largely determined by the amount of milk produced by the cow and also the circulating concentration and sensitivity to insulin. Entry rate is a function of her stored glucose and also gluconeogenic capacity. When cows differ in blood concentrations of glucose on the day of calving and shortly thereafter, this may simply reflect her capacity to store glycogen during the dry period and release it rapidly postpartum. Later, differences in blood glucose may reflect the cow's insulin sensitivity as well as her capacity to acutely adapt to lactation and synthesize a large amount of glucose within liver tissue.

The intriguing feature of the aforementioned studies of blood glucose is that the authors were describing relationships between blood glucose and pregnancy when the insemination was occurring several weeks after the differences in blood glucose. The suggestion is that the early postpartum metabolic profile that includes blood glucose concentrations is predictive of subsequent postpartum fertility.

Mechanisms Through Which Early Postpartum Glucose Can Affect Reproduction

Inadequate blood glucose during early lactation theoretically compromises the function of tissues that depend on glucose as a substrate for carbon skeletons and intracellular energy supply. Metabolites such as NEFA and BHBA, as well as the hormones insulin and IGF1, all of which are controlled by glucose, may also play a role in controlling tissue function. The first 30 days postpartum is a

critical time for the cow with respect to the impact that metabolites and metabolic hormones can have on reproduction. Two essential processes that may be directly affected by glucose, the restoration of ovarian cyclicity and uterine involution, will be discussed.

Restoration of Ovarian Cyclicity Postpartum

The bulk of the research performed about metabolites and metabolic hormones in postpartum cows has focused on the re-initiation of ovarian cyclicity. There is a positive association between insulin, IGF1, and the day postpartum that the cow begins to cycle (Velazquez et al., 2008). LeRoy et al. (2008) concluded that glucose and insulin were the most likely molecules to exert an effect on hypothalamic gonadotropin releasing hormone (GnRH) secretion in the postpartum dairy cow. Increasing glucose supply so that both circulating insulin and IGF1 are increased, therefore, should theoretically cause an earlier resumption of cyclicity postpartum by causing the cow to release more GnRH and have more luteinizing hormone (LH) in the system, which is stimulatory to the ovary. There is also strong synergism for insulin, IGF1 and LH at the ovarian level that shortens the interval to first postpartum ovulation (Kawashima et al., 2012; Lucy 2011).

Uterine Health and Immune Function

Great emphasis is now placed on uterine health and the central place that uterine immune cell function occupies in determining the reproductive success of the postpartum cow (LeBlanc, 2012). Under normal circumstances, uterine involution is completed during the first month postpartum. During involution, the uterus shrinks in size, re-establishes the luminal epithelium, and immune cells (primarily polymorphonuclear neutrophils or PMN) infiltrate the uterus to clear residual placental tissue as well as infectious microorganisms (LeBlanc, 2012).

The postpartum cow has a depressed immune system particularly during the first month after calving. The current theory is that the metabolic environment in postpartum cows suppresses the innate immune system through effects on PMN function (LeBlanc, 2012). In most cases, changes in circulating concentrations of nutrients and metabolites that occur in the postpartum cow are exactly opposite to those that would benefit the function of PMN. For example, glucose is the primary metabolic fuel for PMN (Moyes, 2015). The glucose is stored as glycogen within the PMN. Galvão et al. (2010) observed that cows developing uterine disease had less circulating glucose and lower glycogen concentration in their PMN. Their conclusion was that the lower glycogen reserve led to a reduced capacity for oxidative burst in PMN that predisposed the cow to uterine disease. There is good agreement between in vitro analyses of PMN function and epidemiological evidence that indicates

that an abnormal metabolic profile during the periparturient period predisposes the cow to uterine disease during the early postpartum period and infertility later postpartum (Chapinal et al., 2012).

When is the Metabolic Profile Affecting Immune Function Established?

In their work in which an index for physiological imbalance was created, Moyes et al. (2013) concluded that an index that included NEFA, BHBA, and glucose was predictive of postpartum uterine disease especially when the prepartum index was used. In all likelihood, the metabolic profile associated with uterine disease is initiated before or shortly before calving. This is not surprising given the relatively acute nature of the physiological events at the time of calving and the homeorhetic mechanisms at the initiation of lactation. A cow's homeorhetic capacity (i.e., capacity for gluconeogenesis, lipid mobilization, etc.) and her inherent resistance to disease are largely manifested after calving, but the underlying biology is theoretically in place before she calves.

▪ Blood Glucose Concentrations Later Postpartum (During the Breeding Period)

Assuming that uterine involution is complete and the cow has begun cycling, what are the implications of the metabolic profile of the cow during the breeding period? The metabolic profile of the later postpartum cow (greater than 30 days postpartum) still involves relatively low concentrations of glucose, insulin, and IGF1, although concentrations of NEFA and BHBAA have typically normalized.

Estrous Cyclicity During the Breeding Period

Patterns of estrous cyclicity for lactating cows are less regular compared with the estrous cycle of nulliparous heifers (Remnant et al., 2015). The hormonal environment created by lactation (in this example, low blood glucose, insulin and IGF1 concentrations) may potentially affect the capacity for ovarian cells to respond to gonadotropins (FSH and LH). In the cycling cow, this could potentially affect estradiol production by the follicle as well as progesterone production by the corpus luteum. Low blood glucose could potentially compromise a variety of essential metabolic processes in ovarian cells including the oocyte that depends on glucose for energy. There is also the potential for greater steroid metabolism in lactating compared with nonlactating cows that can be explained by greater dry matter intake in cows that are lactating. Lower circulating estradiol from the preovulatory follicle can lead to abnormal patterns of follicular growth, anovulatory conditions, multiple ovulation and reduced estrous expression.

Glucose as a Substrate for the Developing Embryo and Fetus

Glucose is typically thought of as a key energy source for ATP production through mitochondrial oxidative phosphorylation. Glucose is not used primarily for metabolic fuel production, however, by either the mammary gland or the pregnancy. In the mammary gland, the bulk of the glucose is used to produce lactose. Likewise, in the uterus and placenta the bulk of the glucose is used to supply carbons for the synthesis of cellular components (nucleotides, amino acids, lipids, etc.). This latter phenomenon is known as the "Warburg effect" and typifies proliferating cells.

In a study performed by Green et al. (2012), the major conclusion was that for a given day of pregnancy, the fetus and placenta from a lactating cow were smaller (weighed less) than the fetus and placenta from a nonlactating cow. Less glucose reached the fetus in a lactating compared with a nonlactating cow, perhaps because maternal glucose concentrations were lower during lactation (Lucy et al., 2012). The reduction in glucose reaching the pregnancy can potentially affect how the pregnancy develops because the pregnancy depends on glucose as a substrate for tissue synthesis and metabolic energy.

A recent study in dairy cows demonstrated that pregnant cows that undergo pregnancy loss have lower blood concentrations of pregnancy-associated glycoproteins (PAG) leading up to the time that the pregnancy is aborted (Pohler et al., 2015). The lower blood PAG concentration may indicate that the cow is pregnant with a small embryo or fetus. Perhaps this small embryo or fetus is created when the cow has inadequate glucose or growth factor concentrations.

We recently completed 2 separate studies where we attempted to correlate blood glucose concentrations as well as a variety of other metabolic indicators with size of the fetus and amnion vesicle (Stratman and Lucy, unpublished). In these studies we only found minimal effects of circulating blood glucose, insulin, and IGF1 concentrations on the development of the pregnancy. Our conclusion was that the conceptus is fully capable of developing in a low glucose and growth factor (insulin and IGF1) environment that typifies the cow after 100 days postpartum. Other factors must lead to poor embryonic development and embryonic loss in lactating dairy cows.

▪ Practical Methods to Increase Glucose Supply Postpartum

Glucose is a difficult molecule to study postpartum because of the numerous homeorhetic mechanisms that tightly control its concentration. Two cows with similar blood glucose may have a vastly different metabolic profile (BHBA,

NEFA, insulin, IGF1, and insulin resistance). The fastest and most dependable method to change blood glucose concentrations is reduce demand, for example, by changing milking frequency (3 times daily to 2 or 1 time daily; Stelwagen et al., 2013). Reducing the milking frequency may not be practical or economical for most dairies. Alternative methods for improving circulating blood glucose concentrations postpartum begin during the dry period and extend into early lactation.

Fewer or Zero Days Dry

Cows that do not have a dry period produce less milk. They also have improved metabolic status postpartum as indicated by lower NEFA and greater glucose, insulin, and IGF1 concentrations (Chen et al., 2015; Jolicoeur et al., 2014). However, van Knegsel et al. (2013), in their review of the literature, concluded that the evidence for improved reproduction in cows with a reduced dry period was inconsistent. The greatest benefit to reducing dry period length may be in the prevention of over-conditioned dry cows that have an undesirable metabolic profile postpartum.

Appropriate Dry Cow Feeding and Nutrition

Dry cow nutrition is essential for maintaining a healthy liver postpartum and maintaining good reproduction (Drackley and Cardoso, 2014). Cows that are overweight (BCS >3.75) at calving will develop fatty liver. The inflammation associated with fatty liver inhibits liver metabolism and gluconeogenesis (Garcia et al., 2015). Cows with fatty liver are incapable of achieving the high rates of gluconeogenesis that are needed to maintain adequate glucose supply (McCarthy et al., 2015c). Inadequate glucose supply leads to ketosis and negative downstream effects on reproduction (LeBlanc, 2015).

Treating ketotic cows with propylene glycol will elevate glucose and normalize BHBA (Bjerre-Harpøth et al., 2015; Piantoni and Allen, 2015). The improvements in reproduction that are observed after ketosis treatment demonstrate the important relationship between metabolite concentrations early postpartum and subsequent reproductive function (LeBlanc, 2015).

Less is known about appropriate methods to achieve a large glycogen supply in liver and muscle at calving. Although glycogen is typically depleted rapidly postpartum, a larger glycogen store could perhaps enable the cow to achieve greater blood glucose concentrations in the short term postpartum as is seen for cows with better reproduction. Although it is tempting to think that increasing energy prepartum will increase glycogen stores, this feeding strategy typically leads to overweight cows that are predisposed to fatty liver.

Postpartum Starch and Monensin

A logical approach to address inadequate glucose supply postpartum is to feed starch and monensin. Starch hydrolysis in the rumen and small intestine yields glucose and a greater proportion of propionate (relative to acetate) that can be used to synthesize glucose via gluconeogenesis in liver. Monensin feeding also increases the amount of propionate produced by the rumen which will also support the synthesis of glucose via gluconeogenesis.

In general, an effect of monensin is seen on BHBA (reduced) (Duffield and Bagg, 2000). Cows fed monensin have a greater capacity to convert propionate to glucose (via gluconeogenesis) which could explain the reduction in BHBA (McCarthy et al., 2015b). Starch feeding also reduces BHBA and may increase glucose and insulin concentrations (McCarthy et al., 2015b). These improvements in glucose and insulin associated with starch and monensin feeding have been linked to improved immune cell function in one study (Yasui et al., 2016).

Although the effects of starch feeding and monensin on postpartum metabolites have been demonstrated, there is less information concerning the reproduction in these cows. Dyck et al. (2011) reduced interval to first ovulation by feeding starch but did not show additional improvements in reproduction. Their data were similar to Gong et al. (2002) who showed a shorter interval to first ovulation in cows fed a diet designed to increase blood insulin concentrations postpartum. In their meta-analysis, Duffield et al. (2008) concluded that monensin feeding in postpartum cows reduced the risk of ketosis but had no effect on first service conception.

Starch and monensin generally normalize herd-level metabolic indicators but reproduction is not necessarily improved. When ketotic cows, however, are treated there is clearly a benefit to reproduction (LeBlanc, 2015). This probably indicates that when cows are metabolically balanced postpartum (appropriate concentrations of NEFA and BHBA) there is no benefit to reproduction through normalizing further their metabolite concentrations by additional starch or monensin feeding. This statement does not negate other benefits of monensin feeding that include increased milk production (McCarthy et al., 2015a). In cows that are metabolically imbalanced as evidenced by ketosis, there is a benefit to normalizing NEFA and BHBA concentrations with respect to improving postpartum reproduction.

▪ Conclusions

The endocrine and metabolic environment of the lactating cow affects the capacity of the cow to become pregnant postpartum. There is ample evidence that the hormones responsible for the homeorhetic mechanisms that support

lactation can also act on the uterus and ovary to affect their function prior to and during the breeding period. In addition to the hormonal environment, the metabolic environment created by lactation that includes low blood glucose and elevated NEFA and BHBA impinges upon the ovary as well as the immune system that plays a critical role in restoring uterine health in the postpartum cow. Glucose controls many aspects of the system. Postpartum reproduction in ketotic cows clearly benefits from treatments designed to normalize blood glucose and correct ketosis. Feeding strategies that are designed to increase glucose supply at the herd level (starch and monensin) will reduce BHBA but may not necessarily improve reproduction for the entire herd. This may be because the majority of the cows adapt to early lactation successfully and function within acceptable norms for glucose, NEFA, BHBA and IGF1. Optimizing all aspects of herd nutrition beginning with the dry period is the best way to maintain adequate glucose supply so that postpartum reproduction is not compromised.

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Can Feeding Fats Improve Reproductive Performance in Dairy Cows?

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▪ Take Home Messages

‣ Growing evidence indicates that feeding fat to dairy cows improves their reproductive performance.

‣ Polyunsaturated fatty acids (PUFA) have important beneficial effects on several reproductive functions.

‣ Feeding omega-3 PUFA sources such as flaxseed and fish oil reduced pregnancy losses in dairy cows in several studies.

‣ New research indicates that exposure to omega-3 PUFA enhances early embryo development through differential activation of genes regulating cellular function and proliferation.

‣ Many questions still remain on the optimization and economics of dietary fats and fatty acids to improve dairy cow reproductive function.

▪ Introduction

Poor reproductive efficiency of dairy cows continues to be a challenge for the dairy industry (Ambrose and Colazo, 2007; Leblanc, 2005; Lucy, 2001). Embryonic losses are significant contributors to poor reproductive efficiency in dairy cows, with post-fertilization losses estimated to be up to 60% (Santos et al., 2004).

Nutritional management is one of the strategies available to improve reproduction in dairy cows. There is growing evidence that supplementing fats in dairy rations has beneficial effects on reproductive function and

performance of lactating dairy cows. A recent report (Rodney et al., 2015), adopting the meta-analysis approach, screened over 5000 research papers on the subject of feeding fats and reproductive function in dairy cattle but found only 17 studies with 26 dietary comparisons that were suitable for inclusion in the meta-analysis. Based on their meta-analysis, the authors concluded that feeding fats during the transition period has a positive effect on fertility, with a 27% increase in the probability of pregnancy to service. In addition, cows fed fats during the transition period had a reduction in the interval from calving to pregnancy (9 of 11 comparisons), and a tendency to increase milk production (16 of 23 comparisons).

How dietary fats improve reproductive performance in dairy cattle is not fully understood, but several hypotheses exist (Staples et al., 1998). Improved fertility may result from (a) improvement in energy status, shortening the interval from calving to first postpartum estrus, (b) increased production of steroid hormones, e.g., progesterone, which is essential for pregnancy maintenance, (c) alterations in serum insulin concentrations, which could improve ovarian follicular development, and (d) reduced synthesis and release of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) from the uterus by specific long-chain fatty acids, creating a conducive environment for early embryo survival and pregnancy establishment. Polyunsaturated fatty acids (PUFA) such as linoleic acid, α-linolenic acid, eicosapentaenoic acid and docosahexaenoic acids have garnered much attention lately for their positive effects on reproductive function in cattle, and several excellent reviews have been written on this topic (Mattos et al., 2000; Santos et al., 2008; Wathes et al., 2013). Diets enriched in n-3 PUFA can have positive effects on the development of the early embryo, potentially through the differential activation of genes involved in embryonic cellular growth and proliferation (Salehi et al., 2016).

▪ Fats and Fatty Acids

One of the reasons for feeding fats to dairy cows is to increase energy intake efficiently, because of the higher caloric content packed in fats compared to other feed ingredients. Although both plant (e.g. oilseeds) and animal fats (e.g. tallow, fishmeal) have been used to supplement dairy cow rations primarily to improve energy, many studies have shown beneficial effects of dietary fat on reproductive function (Rodney et al., 2015). Such benefits could be independent of improved energy status, through the action of certain long chain fatty acids on the reproductive axis (Staples et al., 1998). Animals cannot synthesize essential fatty acids such as linoleic and α-linolenic acids; hence, these fatty acids must be made available through diets. Specifically, linoleic (C18:2, n-6) and α-linolenic (C18:3, n-3) acids get converted to very long chain fatty acids through processes of desaturation and elongation. For example, linoleic acid gets converted to arachidonic acid (C20:4, n-6), a precursor for prostaglandins of 2-series, such as $PGF_{2\alpha}$, which plays a major role in the regulation of the estrous cycle, regression of corpus luteum and

termination of pregnancy in cattle. Alpha-linolenic acid gets converted to eicosapentaenoic (C20:5, n-3) acid and docosahexaenoic (C22:6, n-3) acids, precursors for prostaglandins of 3-series (e.g. PGE_3), which can alter PGE:PGF ratios, potentially diminishing the luteolytic effects of $PGF_{2\alpha}$. Moreover, eicosapentaenoic and docosahexaenoic acids can directly act at the level of the uterus (site of $PGF_{2\alpha}$ production) to suppress $PGF_{2\alpha}$ release by reducing the availability of arachidonic acid. Therefore, supplying dietary n-3 PUFA that alter $PGF_{2\alpha}$ release could be a strategy to improve embryo survival in cattle.

Understanding the influence of diets enriched in omega-3 (n-3) and omega-6 (n-6) PUFA on reproductive function in dairy cows has been of interest to us for many years. In this paper, we present research findings on the effects of dietary PUFA, mainly from work done in our laboratory, drawing parallels from related studies done elsewhere, where relevant.

▪ Effects of Dietary Fat (n-3 PUFA) Inclusion During Postpartum Period on Conception Rates and Pregnancy Losses

Flaxseed is one of the richest sources of α-linolenic acid, an n-3 PUFA. In recent years, we have conducted 3 studies investigating the effects of adding flaxseed as an n-3 PUFA supplement in postpartum dairy cow rations on conception rates and pregnancy losses.

Study 1. Alberta

In early work (Ambrose et al., 2006), we compared the effects of type of PUFA on ovarian function, early embryonic survival, conception rates and pregnancy losses in dairy cows receiving a diet supplemented with rolled flax seed (high in α-linolenic acid, n-3 PUFA) vs. sunflower seed (high in linoleic acid, n-6 PUFA). Cows in the study were housed in tiestalls, fed individually and milked twice daily. We randomly assigned 121 lactating dairy cows to 1 of 2 diets starting at approximately 55 days after calving. Diets were isocaloric and contained ~9% rolled oilseeds (either flax or sunflower) on a dry matter basis, providing approximately 750 g of oil per cow per day. The experimental diets were fed for a minimum period of 8 weeks. In a subset of 16 cows (8 per diet), ovarian follicular dynamics were monitored every other day from the day of insemination (d 0) until day 21. To remove the variations associated with estrus detection, timed artificial insemination (TAI) was performed on all cows following a Presynch/Ovsynch protocol. Presumptive conception rate at day 24 was assessed based on progesterone measured in blood (plasma) at 0, 21 and 24 days after TAI. Cows were presumed conceived on day 24 if progesterone concentration had been lower than 1 ng/mL on day 0 (i.e. at

TAI) and greater than or equal to 1 ng/mL on day 21 and 24. Pregnancy diagnosis was performed by ultrasonography 32 days after TAI. Cows confirmed pregnant at 32 days after TAI stopped receiving the experimental rations, whereas non-pregnant cows were placed on the Ovsynch protocol for a second time and rebred by TAI 10 days later. In these cows, the experimental diets continued until pregnancy diagnosis, 32 days after second TAI.

The numbers of small, medium and large ovarian follicles were not affected by diets but the ovulatory follicle before first TAI was larger (16.9 vs. 14.1 mm) in cows fed flaxseed compared to those fed sunflower seed. Presumptive conception rate 24 days after TAI was higher in cows fed flax than in those fed sunflower seed (72.9 vs. 47.5%; P<0.01).

Conception rate: Conception rate to first TAI, confirmed by ultrasound 32 days after TAI, tended to be higher in cows fed flaxseed than in cows fed sunflower seed (48.4 vs. 32.2%; P<0.07). Conception rates to the second TAI and cumulative conception rates (combined for both TAI) were not different between diets.

Pregnancy loss: Cumulative pregnancy loss (from 32 days until calving) in cows fed the flaxseed diet was significantly lower than in cows fed the sunflower seed diet (9.8 vs. 27.3%). In other words, 90.2% of pregnant cows calved in the flax group compared to only 72.7% in the sunflower group.

To determine if the above findings of increased conception rates and reduced pregnancy losses in flaxseed-supplemented rations were repeatable, we conducted 2 independent studies in Oregon and British Columbia. In both studies, lactating Holstein cows were assigned to receive a total mixed ration (TMR) containing 6% fat on a dry matter basis. Each cow received ~2.2 kg of flaxseed per day (flax) or a no-flaxseed supplement (control).

Study 2. Oregon

This work was done in collaboration with the Oregon State University in a large (1300 cow), high-producing commercial dairy herd, with a rolling herd average of 11,435 kg. We randomly assigned 303 early postpartum cows to receive a TMR supplemented with (n=156) or without (n=147) rolled flaxseed. The flax ration was formulated to supply 750 grams of oil per cow per day from rolled flaxseed. The control ration supplied an equal amount of fat from a combination of corn dried distillers grain and solubles, High Fat Product (Archer Daniels Midland Co) and Megalac[®]. Cows were fed freshly mixed TMR twice daily and milked 3 times daily. Diets began approximately 32

days after calving and continued for 31 days after TAI, which occurred following a Presynch/Ovsynch protocol. Cows were subjected to TAI by 1 of 2 technicians, with TAI occurring $\geq$28 days after initiation of experimental diets. Pregnancy diagnosis was performed by ultrasonography 31 days after TAI. Cows diagnosed pregnant were rechecked at 94 days to assess pregnancy losses between 31 and 94 days of gestation.

Conception rate: The conception rate was numerically lower in cows fed flaxseed compared to those fed the control diet, both at 31 days (28.2 versus 42.9 %; P= 0.13) and 94 days (25.6% versus 36.7%; P=0.31). Pregnancy rate at 31 days was influenced (P<0.02) by AI technician (24.4 versus 40.5% for the two technicians) but there was no diet-by-technician interaction.

Pregnancy loss: Overall, 12.2% of the pregnancies were lost between 31 and 94 days of gestation. Though not statistically different, the proportion of pregnancy loss was 37% lower (P= 0.20) in cows fed flaxseed (9.0%; 4 of 44) than in cows fed the control diet (14.3%; 9 of 63).

Study 3. British Columbia

The next study was conducted in collaboration with the University of British Columbia at the Dairy Education and Research Centre in Agassiz, BC. We randomly assigned 266 lactating dairy cows to receive either a TMR supplemented with rolled flaxseed (n=141) or a control ration with no-flaxseed (n=125). As in the previous studies, the flaxseed ration was formulated to provide 750 g of oil per cow per day from rolled flax seed. The control diet was formulated to provide 750 g of fat supplied from tallow and Megalac®. Diets began a minimum of 28 days before TAI and continued until pregnancy diagnosis at 35 days after TAI. Reproductive management procedures were similar to that of the previous study, except that pregnancy was confirmed by ultrasonography at 35 days after TAI, and reconfirmed by rectal palpation at 90 days of gestation. Data on calving were also collected from this location. Early pregnancy loss between 35 and 90 days of gestation, and cumulative pregnancy loss between 35 days and term (calving) were determined.

Conception rate: Diets did not affect the conception rate at 35 days, with flax and no-flaxseed cows averaging 43.3 and 41.6%, respectively. Conception rates at 90 days for the 2 dietary treatments were 40.4 and 38.4%, respectively.

Pregnancy loss: Pregnancy losses between 35 days of gestation and calving were numerically lower (P=0.20) in cows fed flaxseed (8.3%) than in cows fed a control ration (16.3%).

Other Related Studies with Postpartum n-3 PUFA Diets on Conception Rate and Pregnancy Loss

In a Quebec study, Petit and Twagiramungu (2006) assigned 3 groups of 46 cows each to 1 of 3 isonitrogenous, isoenergetic, and isolipidic supplements based on either whole flaxseed (flax), Megalac® or micronized soybeans (soybeans). Rations contained ~10.6% whole flaxseed and ~7.3% total fat on a dry matter basis. The experimental diets were fed from calving until 50 days of gestation in cows that conceived to first AI, or until 120 days for those found not pregnant after the first AI.

Conception rate and pregnancy loss: Conception rates did not differ among treatments, with first service conception rates of 44.4, 55.9 and 40.0%, respectively, for flax, Megalac® and soybean diets. Total embryo mortality was lower (P=0.07) for cows fed flax (0%) compared to those fed either Megalac (15.4%) or soybean (8.0%). Progesterone concentrations were higher from day 17 to 21 of an estrous cycle in cows fed flax. The authors concluded that pregnancy losses could be reduced by feeding whole flaxseed through possible modulations in concentration of progesterone and size of the corpus luteum.

In a large study at the University of Florida, Silvestre et al. (2011) assigned 1380 Holstein cows to diets containing calcium salts of either palm oil (high in saturated fatty acids) or safflower oil (high in linoleic acid) from 30 days before calving until 30 days after calving, and then to receive either calcium salts of palm oil or fish oil (high in eicosapentaenoic and docosahexaenoic acids) from 30 to 160 days postpartum. The experimental diet combinations fed during the transition (-30 to +30 d) and breeding periods (30 to 160 days postpartum), respectively, were palm oil + palm oil; palm oil + fish oil, safflower oil + palm oil, and safflower oil + fish oil. Total dietary fat (dry matter basis) in the breeding period was about 5%, with 1.5% being supplemental fat from calcium salts of either palm oil or fish oil.

Conception rate and pregnancy loss: Although pregnancy per AI at 32 and 60 days after first AI was not affected by diets, pregnancy loss was significantly (P<0.01) reduced in cows fed fish oil versus palm oil (6.0 vs. 11.8%) during the breeding period, regardless of whether the cows were fed diets supplemented with palm or safflower oils during the transition period.

Summary of findings: When n-3 PUFA of either flaxseed or fish oil origin was included in the postpartum rations, conception rate (pregnancy per AI)

did not consistently increase. In 2 of the 5 studies, conception rate tended to increase in cows fed n-3 PUFA, whereas in the other 3 studies, conception rate was not affected by diet; however, pregnancy loss was consistently lower in cows fed diets enriched in n-3 PUFA in all 5 studies (Figure 1). Mean pregnancy losses in the n-3 PUFA dietary group were significantly lower or tended to be lower in 3 of the 5 studies, and numerically lower in 2 studies. These findings strongly suggest that PUFA diets of flaxseed or fish oil origin (predominantly n-3 fatty acids) can reduce pregnancy losses in dairy cows.
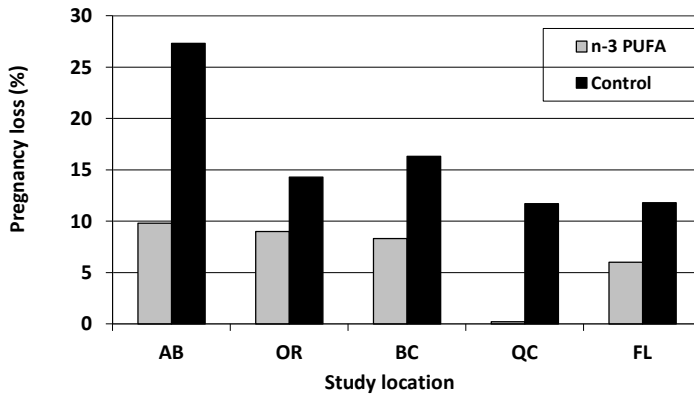
Figure 1. Pregnancy loss in 5 different studies in which lactating dairy cows were fed a diet enriched in n-3 PUFA of either flaxseed or fish oil origin vs. a control diet containing low or no n-3 fatty acids. Study locations were Alberta (AB), Oregon (OR), British Columbia (BC), Quebec (QC) and Florida (FL). The total number of cows in each study and probability of difference for pregnancy losses in AB, OR, BC, QC and FL were 121 (P<0.05), 303 (P>0.10), 263 (P>0.10), 138 (P=0.07), 1380 (P<0.01).

▪ Effects of Dietary Fat Inclusion During the Prepartum Period on Postpartum Reproductive Performance

Study 1. (Colazo et al., 2009)

This study was designed to determine the effects of feed restriction and source of dietary fatty acids during the close-up dry period on postpartum reproductive performance of dairy cows. We hypothesized that (1) restricted feed intake during the prepartum period will improve postpartum intake and reduce negative energy balance, thereby contributing to improved reproductive performance, and (2) that inclusion of oilseeds with different fatty acid profiles in prepartum diets will have a differential influence on resumption of cyclicity and carryover beneficial effects on fertility.

We assigned 72 cows to receive 1 of 6 diets starting 34 days before expected calving date in a 2 x 3 factorial arrangement. Dietary treatments were 2 levels of feed intake (ad libitum; AL or 24% feed restriction; FR), and 3 types of rolled oilseed supplements: canola, linola or flax at 8% of dry matter, to enrich the diets with oleic, linoleic or α-linolenic acids, respectively. A common lactation diet containing no oilseeds was fed to all cows after calving. Reproductive tracts were examined by ultrasonography twice weekly from 7 days after calving until confirmation of first ovulation. After an elective waiting period of 65 days, 66 of the 72 cows were subjected to Ovsynch/TAI and pregnancy was diagnosed 32 days after TAI.

The mean dry matter intake during the prepartum period of cows fed AL (11.9 kg/d) was higher than that of FR cows (9.0 kg/d). Prepartum energy balance was higher in AL than in FR cows (3.6 vs. -0.4 Mcal/d; P<0.01) but fatty acid source (i.e., oilseed) did not affect energy balance. Milk production was not affected by FR but affected by the source of dietary fatty acids with canola, linola and flax fed cows yielding 32.9, 36.4 and 34.6 kg, respectively. The birth weight of calves was not affected by level of intake or fatty acid source. A significantly higher proportion of cows in the AL group suffered uterine infections relative to FR (27 vs. 6%; P<0.01) but FR cows tended to have a higher incidence of ovarian cysts (20 vs. 5%; P =0.09). Feed restriction during the close-up dry period had a negative impact on conception to first TAI (19 vs. 47%; P=0.02) compared to AL, without affecting the interval from calving to uterine involution or ovarian function. However, the mean interval from calving to first ovulation was longer (P =0.02) in cows fed canola seed (35 d) compared to those fed either linola (24 d) or flax seed (21 d). Pregnancy to first AI, cumulative pregnancy from 75 to 280 days postpartum, and days open were not affected by oilseed type (Table 1) indicating that feeding fats in the prepartum period has little carryover beneficial effects in terms of reproductive outcomes, although feeding oilseeds high in PUFA (e.g. linola, flax) shortened the interval from calving to first ovulation.

Table 1. Postpartum reproductive performance indices in cows fed canola, linola or flaxseed during the prepartum period (Colazo et al., 2009)

Index	Prepartum diet			*P*
	Canola	Linola	Flax	
Cows (n)	24	24	24	
Calving to 1st ovulation[1] (d)	34.7 ± 3.1	23.7 ± 3.2	21.0 ± 3.1	0.02
Pregnancy to 1st TAI (%)	26.3	43.5	29.2	0.43
Cumulative pregnancy[2] (%)	54.2	70.8	62.5	0.49
Days open	186 ± 10.9	167 ± 14.3	166 ± 14.5	0.24

[1]Canola > linola and flax
[2]Pregnancies from 75 to 280 d postpartum

Study 2. Salehi et al (2016)

The finding in our previous study (Colazo et al., 2009) that the interval from calving to first ovulation was shorter in cows fed diets supplemented with linola (high in linoleic acid) or flax seed (high in linolenic acid) than in those fed diets supplemented with canola seed (high in oleic acid) prompted us to investigate further. As canola seed is a common ingredient in dairy cattle rations, we set out to confirm our previous observations and further investigate the effect of dietary canola seed on reproductive function. In our earlier study (Colazo et al., 2009), we did not have a control diet with no supplemental fat; therefore, we also included a control (no added fat) ration in this study.

We hypothesized that cows given a prepartum diet supplemented with canola seed will have a longer interval from calving to first ovulation, as observed previously, compared to those fed diets supplemented with no-oilseed or sunflower seed. Due to difficulties in sourcing linola seed, we used sunflower seed instead because of its high linoleic acid content (73%), comparable to that of linola (72%). Our objectives were to determine the effects of supplemental fat (no-oilseed vs. oilseed) during late gestation and the source of fat (canola vs. sunflower seed) on intake, milk production and composition, calf birth weight, postpartum health disorders, ovarian function and reproductive performance in dairy cows. Pregnant Holstein cows blocked by parity and body condition were assigned to 1 of 3 diets containing rolled canola seed (high in oleic acid; n = 43) or sunflower (high in linoleic acid; n = 45) at 8% of dry matter, or no-oilseed (control; n = 43), for the last 35 days of gestation. All cows received a common lactation diet after calving. Ovarian ultrasonography was performed twice weekly to monitor follicular growth and to determine the interval from calving to first ovulation.

Prepartum oilseed supplementation, more specifically sunflower seed, increased postpartum intake in primiparous cows without affecting prepartum intake or milk yield. On the contrary, in multiparous cows, prepartum oilseed supplementation decreased intake both pre and postpartum, and milk yield during the first 2 weeks. Regardless of parity, prepartum diet containing canola seed reduced postpartum feed intake compared to those fed sunflower seed. Mean nonesterified fatty acids (NEFA) concentrations at week -3 were greater in cows given supplemental oilseed than those given no-oilseeds.

Gestation length (276 vs. 273 d) and calf birth weight (43.7 vs. 41.0 kg) were increased in cows given supplemental oilseed prepartum compared to those fed no-oilseed. Interestingly, a disproportionate increase in the birth weight of female calves was evident in cows fed oilseed (43.9 vs. 40.4 kg; P=0.02). The type of oilseed also had a differential effect on calf birth weight with female calves born of cows fed sunflower being heavier than female calves born of

cows fed canola seed (45.4 vs. 42.3 kg; P=0.08). Reproductive disorders tended to be greater in cows fed supplemental oilseed than those fed no-oilseed (42 vs. 23%). Furthermore, cows fed sunflower seed had greater incidence of dystocia (35 vs. 18%) and total health disorders (52 vs. 32%) than those fed canola seed. Oilseed supplementation did not have any significant effect on ovarian function or fertility (Table 2). More detailed results of this work can be found in our recent paper in the Journal of Dairy Science (Salehi et al., 2016).

Table 2. Postpartum ovarian function and fertility in cows fed control, canola or sunflower seed during prepartum period (Salehi et al., 2016)

| Index | Prepartum diet | | | *P* |
	Control	Canola	Sunflower	
Cows (n)	31	31	33	
Calving to 1st ovulation (d)	20.7 ± 1.6	22.9 ± 1.5	20.6 ± 1.4	0.47
Cows ovulated by 35 d (%)	71.0	77.4	84.8	0.21
Pregnancy to first AI (%)	22.6	25.8	33.3	0.53
Cows pregnant by 150 d[1] (%)	41.9	51.6	42.4	0.78
Cows pregnant by 250 d[2] (%)	58.1	74.2	72.7	0.06

[1]Cumulative pregnancies up to 150 d postpartum
[2]Cumulative pregnancies up to 250 d postpartum

Summary of findings: In the first study, the mean interval from calving to first ovulation was longer in cows fed canola seed compared to those fed either linola or flax seed. However, the results were not repeatable in the second study. In the second study, prepartum oilseed supplementation at ~8% reduced intake during the entire experimental period (pre and postpartum) and decreased milk yield during early lactation in multiparous cows. Oilseed supplementation also increased calf birth weight and postpartum health disorders. However, we found no significant differences in postpartum ovarian function and reproductive performance between the 2 prepartum diets.

▪ Effects of Dietary Fat Inclusion on Embryonic Development

Several studies have shown that fats and fatty acids can affect embryonic development in cattle (Tables 3 and 4). Both in vivo and in vitro studies have investigated the role of fats and fatty acids on bovine embryo development. Three such studies conducted in our laboratory are summarized below.

Study 1. (Thangavelu et al., 2007)

Because inclusion of flaxseed in dairy cow rations increased conception rates in some studies and reduced pregnancy losses in many studies, we proposed

that reduced pregnancy losses were due to enhanced embryonic development during the early days of gestation in cows fed a diet enriched in α-linolenic acid. We (Ambrose et al., 2006) previously found that lactating dairy cows fed flaxseed had significantly lower pregnancy losses than those fed sunflower seed; however, a no-PUFA control was not included in that study. Therefore, in the study by Thangavelu et al. (2007), we fed 3 diets, 2 high in unsaturated fatty acids and 1 high in saturated fatty acids.

Table 3. Effects of fats and fatty acids on embryo development (in vivo studies)

Source ref.	Type of fat	Main result
Salehi et al., 2014; Reprod Fertil Dev 26:218	-Flax seed -Sunflower seed -Canola seed	Feeding rolled flaxseed reduced the proportion of degenerated embryos.
Moallem et al., 2013; Reproduction 146:603	-Flaxseed oil -Fish oil -Saturated fatty acid	Feeding flaxseed oil enhanced the cleavage rate of in vitro fertilized oocytes and tended to improve blastocyst rate compared to a diet enriched in saturated fatty acid.
Zachut et al., 2010; J Dairy Sci 93:529	-Control -Encapsulated flax oil -Encapsulated sunflower oil	Feeding flax oil increased the cleavage rate of in vitro matured oocytes as compared with those of control cows.
Cerri et al., 2009; J Dairy Sci 92:1520	-Calcium salts of palm oil -Calcium salts of linoleic and trans-octadecenoic acids (LTFA)	Feeding LTFA improved the proportion of excellent-, good-, and fair-quality embryos, and embryos from cows fed LTFA had a greater number of blastomeres than embryos from cows fed palm oil
Childs et al., 2008; Theriogenology 70:992	-Palmitic acid -Rumen protected n-3 PUFA	Feeding n-3 PUFA reduced the proportion of degenerated embryos.

Table 4. Effects of fats and fatty acids on embryo development (in vitro studies)

Source ref.	Type of fat	Main result
Salehi et al., 2014; WCDS Adv Dairy Technol 26:379	-Serum collected from cows fed flax (vs.) -Commercial fetal calf serum	Adding serum collected from cows fed flax improved development of embryo derived from low quality oocytes.
Adrema et al., 2011; Biol Reprod 85:62	-Palmitic acid -Stearic acid -Oleic acid	Palmitic and stearic acid had detrimental effect on oocyte developmental competence, whereas oleic acid improved oocyte developmental competence and blastocyst rate.
Marei et al., 2010; Reproduction 139:979	-Control -Linoleic acid	Adding linoleic acid inhibited cumulus cell expansion, delayed development of the oocytes to the metaphase II stage and reduced cleavage and blastocyst rate.
Marei et al., 2009; Biol Reprod 81:1064	-Control -α-Linolenic acid	Adding α-linolenic acid to in vitro maturation medium enhanced oocyte maturation and subsequent embryo development.
Leroy et al., 2005; Reproduction 130:485	-Palmitic acid -Stearic acid -Oleic acid	Addition of palmitic or stearic during oocyte maturation had negative effects on maturation, fertilization, and subsequently cleavage rate and blastocyst yield.

We hypothesized that feeding flaxseed (n-3 PUFA) will enhance early embryonic development compared to sunflower seed (n-6 PUFA) or saturated fatty acids. The objective was to compare embryonic development (as determined by the number of blastomeres, i.e., cells of the early embryo) in cows fed rations supplemented with saturated fatty acids or unsaturated fatty acids (flaxseed or sunflower seed). Twenty-four cyclic lactating Holstein cows (86 ± 22 d postpartum; 3.0 ± 0.4 lactations) were randomly assigned to 1 of 3 dietary groups. Diets were isonitrogenous and estimated energy intake was similar across diets. After receiving the diets for approximately 20 days, cows were subjected to superovulatory treatments and artificially inseminated twice (0800 h and 1900 h) with frozen-thawed semen from a single young sire. Seven days after AI, embryos were collected non-surgically. Transferable (excellent and good) quality embryos from each of the 3 dietary groups were

stained and blastomere nuclei counted using a microscope and automated software.

Total ova and embryos, or transferable embryos, did not differ among the diets, but the overall recovery rate, defined as embryos/ova recovered as the proportion of corpora lutea, was higher in cows of the sunflower group. Fertilization rate was also not affected by diets. Total blastomere number was affected by diet ($P<0.01$; Table 5). When all categories of embryos were considered, embryos collected from cows fed saturated fat had fewer blastomeres than those from cows fed flax or sunflower seed. The differences were clearly evident in the expanded blastocyst stage, where embryos of cows fed flax or sunflower seed had a greater number of blastomeres than those from cows fed saturated fats. Blastomere numbers of expanded blastocysts did not differ between flax and sunflower seed dietary groups.

Table 5.   Mean (± SEM) total number of blastomere nuclei of embryos recovered from cows fed diets supplemented with saturated fatty acid (SAT), flaxseed (FLX) or sunflower seed (SUN). Embryos (n = 61) were collected, non-surgically 7 days after AI, stained, and blastomere nuclei counted under a confocal microscope.

Stage of embryo	Dietary Groups			
	SAT	FLX	SUN	*P*
Morula	64.4 ± 4.1[a]	76.3 ± 4.4[b]	65.6 ± 4.1[a]	0.09
Blastocyst	77.5 ± 6.1[a]	88.6 ± 6.5[ab]	93.7 ± 5.7[b]	0.07
Expanded blastocyst	89.3 ± 9.5[a]	115.4 ± 6.3[b]	132.3 ± 8.3[b]	0.02
All embryo stages[1]	77.1 ± 3.9[a]	93.4 ± 3.4[b]	97.1 ± 3.1[b]	0.01

[1]Includes morula, blastocyst, and expanded blastocyst
[ab]Means with different superscripts within rows differ or tend to differ

Another study (Cerri et al., 2009), conducted jointly by researchers in the universities of California-Davis and Florida, supported the above findings. Holstein cow diets were supplemented with fat (2% of dry matter), either a calcium salt of palm oil (mostly saturated fatty acids) or a calcium salt high in linoleic acid and a blend of trans-octadecenoic acid (mostly unsaturated fatty acids) from 25 days before calving until 70 days postpartum. Cows were inseminated following a Presynch/Ovsynch protocol and embryos collected 5 days after insemination. Approximately 75 cows were assigned to each dietary treatment. The cows that received the mostly-unsaturated fatty acid diet had a higher proportion of excellent, good and fair-quality embryos. In addition, embryos from cows fed the unsaturated fatty acid diet had a greater number of blastomeres than those from cows fed the mostly-saturated fatty acid diet.

Study 2. (Salehi et al., 2013)

We investigated the effects of diets enriched in oleic, linoleic or α-linolenic acid on the development and transcriptomic profile (gene expression) of embryos collected from non-lactating dairy cows. Cows received 1 of 3 diets supplemented with rolled oilseeds (8% of dry matter): flax (n=8), sunflower (n=8) or canola (n=8). After a minimum 35-day diet adaptation, cows were superovulated, artificially inseminated with semen of the same sire and embryos collected 7½ days after AI. Cows fed flax had fewer degenerated embryos compared to those fed either sunflower or canola seed. The proportion of viable embryos was also higher in cows fed flaxseed (Salehi et al. 2013). The transcriptome profile of in vivo produced embryos revealed that 175 genes were differentially expressed in embryos from cows fed flax compared to those fed either canola or sunflower seeds. The differentially expressed genes mainly had roles in cellular growth and proliferation, and lipid metabolism (data unpublished).

Study 3. (Salehi et al., unpublished)

Using a whole animal (in vivo) model, it is not possible to determine whether the effect of PUFA on embryo quality is exerted at the follicular level (on oocytes, before fertilization) or at the oviduct/uterine level (on embryos, after fertilization). Therefore, we used an in vitro model to investigate whether fatty acids specifically influenced post-fertilization development of embryos. Serum collected from cows fed 2 of the above rations (flax and sunflower) was added separately to groups of early-stage embryos produced by in vitro fertilization of oocytes harvested from slaughter-house ovaries. Serum was added to the medium used for post-fertilization culture so that embryos were exposed to either of the serum treatments for up to 7 days. Excellent and good quality embryos from each treatment group were used for gene expression studies. Adding serum collected from cows fed flaxseed compared to those fed sunflower seed increased the expression of genes responsible for cell proliferation and differentiation as well as genes involved in maternal recognition of pregnancy without affecting morphological development.

Summary of findings: Collectively, our results and those from other researchers indicate that fats and fatty acids can influence early embryonic development. When compared to n-3 and n-6 PUFA, saturated fatty acids seem to exert a detrimental effect on embryos. In contrast, n-3 PUFA have a positive effect on early embryo development, including differential expression of genes that favour cell proliferation and pregnancy recognition.

▪ Conclusions

Getting back to our original question regarding whether feeding fats can improve reproductive performance in dairy cows, although there are inconsistences among reports, data from many studies compared by meta-analysis (Rodney et al., 2015) indicate that feeding fats can improve reproductive performance. Several studies that have used dietary n-3 PUFA (flaxseed/fish oil) in postpartum rations found a consistent reduction of pregnancy losses in lactating dairy cows (Figure 1). There is also a growing body of evidence showing positive effects of PUFA, particularly n-3 PUFA, on embryo development (Tables 3 and 4). All of these findings, and many other positive effects of fats and fatty acids on reproduction reported elsewhere (Ambrose and Kastelic, 2003; Santos et al., 2008; Wathes et al., 2013), strongly suggest that feeding fats high in n-3 PUFA to dairy cows can be beneficial. One of the biggest and most common problems with studies where the measured endpoint is conception rate or pregnancy loss, is the lack of sufficient statistical power due to inadequate animal numbers. Therefore, large multi-location, multi-year field studies are essential to find a more definitive answer to our question. Finally, a major consideration to feeding supplemental fats high in n-3 PUFA is the cost factor. Where practical, selective feeding of n-3 PUFA diets to cows with a high risk of reproductive loss (e.g., multiparous cows, those in poor body condition, etc.) might be a cost-effective approach.

▪ Acknowledgements

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The Importance of BCS Management to Cow Welfare, Performance and Fertility

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▪ Take Home Messages

- ▸ Cows attempt to regulate their body energy reserves to a target BCS during early lactation; thus, cows with greater BCS at calving will lose more BCS in early lactation.

- ▸ Increasing BCS at calving exacerbates negative energy balance in early lactation rather than preventing it.

- ▸ Genetic selection for milk production has decreased the target BCS of cows.

- ▸ Extreme negative energy balance and loss of BCS in early lactation may avoidable.

- ▸ For high producing Holstein cows in North America, BCS at calving should not be greater than 3.0.

▪ Introduction

Dairy cows, like all mammals, store surplus energy not immediately needed in the form of fat (triglycerides) in various adipose tissues throughout the body (Friggens, 2003). The physiological regulation of pregnancy and lactation results in cyclic changes in body fat reserves, as fat is mobilized in early lactation to meet energy demands of increasing milk production and then replenished in mid- to late lactation in anticipation of the next calving and lactation.

Management of body fat content is critical to achieving the sometimes antagonistic goals of good fertility, high milk production, and health. At

present, the best on-farm tool for long-term management of body energy reserves is body condition scoring. Assessment of body condition scores (BCS) in late lactation, at dry-off, at calving, and at initiation of breeding can be helpful in determining whether the nutritional program and other management practices are adequate. Where problems in health, fertility or production are present, evaluation of BCS can help troubleshoot the cause or causes.

The topic of BCS is not new and has been addressed by a number of authors in previous years of this series. Several good scientific reviews are available for the interested reader to find more information (Garnsworthy, 2007; Roche et al., 2009). My objective is to review well-established principles of biology related to BCS, as well as to address some newer aspects of the relationships between BCS and health, fertility and production. In many cases, managers and their advisors overestimate what an optimal BCS at calving should be.

▪ Optimal BCS From the Cow's Perspective: The "Target BCS"

Although BCS is assigned according to different scales around the world, the scale used in Canada and the rest of North America ranges from 1 (emaciated) to 5 (obese). Scorers today usually attempt to assign scores with quarter-point increments. By definition, it would seem that the midpoint (BCS = 3.0) of the scale should be the desired score at the start of the lactation cycle (calving).

There is strong evidence to indicate that the degree of body fatness is regulated to a certain optimum within individual cows. This optimum appears to represent a "target BCS" that cows attempt to reach somewhere between 10 and 20 wk of lactation (Garnsworthy, 2007). The cow's target score is a genetically determined "set point", which allows the cow to produce milk, reproduce and remain healthy. The cow's target BCS should not be confused with management recommendations for optimal BCS based on data or perceptions of managers.

The target BCS for most high-producing Holstein cows is now in the range of 2.0 to 2.5, which has continued to decrease with genetic selection for high milk yield and high yields of milk components (Garnsworthy, 2007). Where management pushes cows away from their optimum score, either too fat or too thin, cows will respond by repartitioning dietary nutrients to restore body fatness to the optimum target BCS. This means that cows that are thin relative to their target score at calving will gain BCS after calving, and cows with excessive BCS will mobilize body fat during early lactation (Figure 1). Such responses have been observed in other studies too, including our own (Douglas et al., 2006).

▪ BCS and Welfare: Associations with Health

As with other mammals, including humans, both excessively thin and excessively fat cows may represent a welfare problem (Friggens, 2003). In most herds in confinement systems and fed TMR, it is rare to see cows in excessively thin BCS unless as a result of illness or lameness. Occasionally, widespread drought or crippling economic conditions might lead to herds being too thin, but usually not to the point of semi-starvation. In grazing systems, such as those in New Zealand and Ireland, declining grass abundance and quality as cows move into winter can result in the herd being too thin for optimum reproduction and production in the next lactation. Thin cows may be more susceptible to infectious disease.
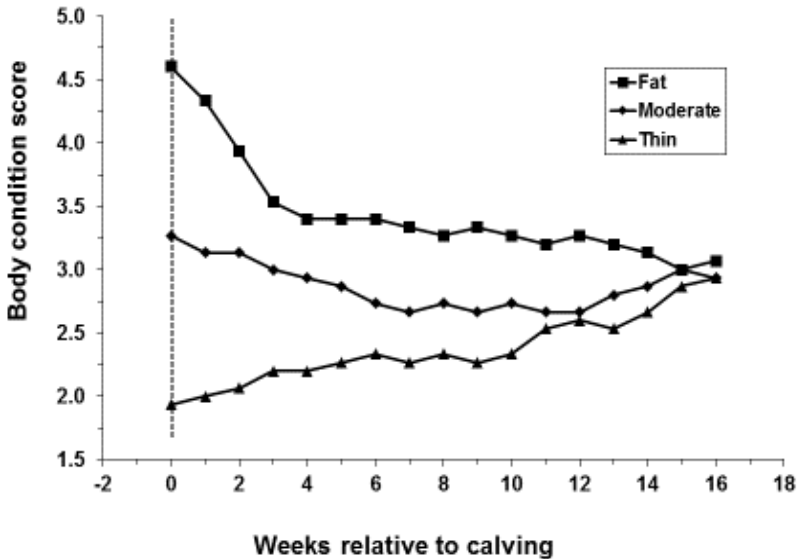
Figure 1. Cows were fed during mid- to late lactation to be fat, moderate, or thin BCS at calving (vertical dotted line). All cows were fed the same lactation ration after calving for ad libitum intake. By 15–16 weeks into lactation all cows had converged at the same BCS. Thin cows produced more milk and consumed more DM than fat cows, with cows of moderate BCS being intermediate. Redrawn from Garnsworthy (2007).

On the other hand, excessive BCS can be common in confinement systems. Improperly balanced diets, poor forage quality that leads to more grain feeding, and poor fertility often lead to cows becoming overconditioned by the next calving. While uninformed consumers may see the thin cow as the most obvious indication of poor welfare, from the standpoint of our common management, the fat cow is generally the greater welfare risk.

The belief that essentially all high producing dairy cows enter negative energy balance (NEB) after calving is deeply engrained in those who work with dairy production. However, as will be shown in a later section, we have known that this is not necessarily the case. Cows that calve with BCS greater than their target will mobilize that BCS in early lactation. The mobilized fat circulates in blood as nonesterified fatty acids (NEFA), which can be used as a diagnostic tool for adequacy of management during the transition period (Ospina et al., 2010). While NEFA mobilization provides fatty acids to make milk fat and may provide metabolizable energy in addition to what the cow consumes for fueling greater milk production, the resulting NEB carries a greater risk of health disorders and is a major cause of poor reproductive success (Butler, 2003; Garnsworthy et al., 2008).

Rapid loss of body fat after calving and into early lactation directly increases the risks of fatty liver and ketosis (Drackley et al., 2005). The liver takes approximately one-third of the mobilized NEFA. During NEB, most of the NEFA are either converted back into triglycerides that accumulate and cause fatty liver or are converted into the ketone bodies such as beta-hydroxybutyrate (BHBA). Recent studies have shown that subclinical ketosis may occur in more than 40% of cows after calving, with the greatest incidence during the first 2 wk after calving (McArt et al., 2012). The NEB represented by high NEFA and BHBA concentrations in cows is associated with greater occurrence of displaced abomasum and ketosis, loss of milk production, and decreased fertility (Chapinal et al., 2012; Ospina et al., 2010). Negative secondary effects of ketosis are more severe if ketosis occurs within the first week post-calving than in the second or later weeks (McArt et al., 2012).

High BCS at calving, and the NEB and rapid loss of BCS that follow after calving, are also associated with increased occurrence of dystocia, retained placenta, metritis, hypocalcemia and milk fever, mastitis, and lameness (Garnsworthy, 2007; Roche et al., 2009). The "fat cow syndrome" is well known to result in a complex of metabolic disorders and infectious disease problems, many of which may be exceptionally difficult to treat and resolve. Evidence indicates that NEB impairs function of cells of the immune system (Lacetara et al., 2005), which likely explains the greater incidence of infectious diseases like metritis and mastitis. Some of this impairment may result from changes in energy metabolites in blood; high NEFA and high BHBA have been shown to negatively affect immune cells, especially when blood glucose is low (Contreras and Sordillo, 2011). Another factor involved may be the increase in oxidative stress caused by the fat mobilization (Bernabucci et al., 2005).

Cows that calve with excessive BCS have poor appetites and lower DMI than their thinner counterparts (Grummer et al., 2004). This may be a result of the cows' biological drive to return to their target BCS. Mechanistically, recent research has shown that high NEFA mobilization may decrease DMI through

increasing the rate of ATP production within the liver, which is part of the "hepatic oxidation theory" established in cows by Michigan State University researchers (Allen et al., 2009). According to this theory, cows that mobilize BCS will have lower DMI; this can result in greater NEB that in turn increases NEFA mobilization and so on. Cows can enter a "death spiral" of decreasing intake and increasing fat mobilization, contributing to the complex of health problems and perhaps accounting for the greater death loss in confinement TMR systems.

▪ BCS and Fertility

Like health issues, both low and high BCS at calving can negatively affect reproductive efficiency. Cows that are thinner than their target BCS may have prolonged periods of postpartum anestrus (Roche et al., 2009). High BCS and the resulting NEB after calving clearly decrease fertility in cows. Although studies have demonstrated a weak and variable relationship between the degree of NEB and impaired fertility, the time to the lowest NEB and the rate of change in NEB are more strongly related (Butler, 2003; Garnsworthy et al., 2008). Detrimental effects of NEB on reproduction include 1) delayed resumption of ovarian cyclicity, 2) impacts on oocyte or corpus luteum "quality", viability, or function (sometimes referred to as "follicular memory"), and 3) development of fatty liver (Drackley and Cardoso, 2014).

In general, reproductive success is better in cows that ovulate sooner after calving (Butler, 2003). In NEB after calving, the pulse frequency of LH release, the size and development rate of follicles, concentrations of estrogen and progesterone, and size of the corpus luteum all are decreased (Garnsworthy et al., 2008). Successful ovulation depends on estrogen production by the dominant follicle, restoration of pulsatile luteinizing hormone (LH) secretion, and responsiveness of the ovary to LH. The state of NEB is associated negatively with reproductive performance in part because it interrupts these 3 factors (Butler, 2003).

Insulin concentrations generally reflect energy status and dietary adequacy. Insulin links the metabolic and reproductive systems by its necessity to increase synthesis of insulin-like growth factor 1 (IGF-1) in the liver in response to elevated concentrations of growth hormone, to increase estrodiol production by the dominant follicle and to increase LH receptors for ovulation and corpus luteum development (Lucy, 2000; Garnsworthy et al., 2008). Lower insulin and IGF-1 during NEB thus may be related to eventual increases in days to first ovulation, first estrus and conception, and decreased rates of conception and pregnancy.

Extreme NEB also may negatively impact oocyte or corpus luteum quality or viability due to reduced concentrations of progesterone and IGF-1. The decrease in these compounds may be a result of increased uptake of NEFA

and BHBA by the ovary and its follicles, particularly when glucose concentrations are low (Drackley and Cardoso, 2014).

Fatty liver is negatively associated with fertility (Drackley et al., 2005), which may be an indirect effect of the extreme NEB in these cows. However, direct negative effects of fat infiltration on reproduction cannot be discounted. Blood flow through the liver may be altered by fat accumulation expanding cell volume and compressing the circulation between cells. Fat accumulation also may decrease the normal ability of liver cells to metabolize or clear reproductive and metabolic hormones (Drackley and Cardoso, 2014), thus altering the normal signaling to reproductive tissues and pituitary.

▪ BCS and Production

Across systems, countries, and climates, the available evidence indicates that milk production is maximized when the calving BCS is approximately 3.5 (Roche et al., 2009). However, in these same studies there was little additional milk response when BCS greater than 3.0. Thus, it appears that a calving BCS of 0.5 to 0.75 BCS unit greater than the proposed cow's target BCS during early lactation (2.0 to 2.5) is adequate for maximal lactation response. Thinner cows have greater DMI, which in turn will support high milk yields as well as restore body fat reserves (Garnsworthy, 2007).

Cows with high BCS at calving will produce milk with greater fat content, which is a result of the mobilized NEFA being directly incorporated into milk fat (Roche et al., 2009). If dietary energy, particularly glucogenic energy, intake is limited, milk protein may be decreased.

▪ Relationships with Dry Period and Transition Management

Research by our group over the last two decades has shown that allowing dry cows to consume a marked excess of energy relative to their requirements results in many changes typical of excessive BCS, even if cows do not appear to be overconditioned (Drackley and Cardoso, 2014). In these studies, cows averaged about 3.0 to 3.25 at calving. Cows fed a high fibre, controlled energy diet to limit intake to near requirements showed a better metabolic profile after calving than cows fed higher energy close-up diets (Beever, 2006; Janovick and Drackley, 2010; Janovick et al., 2011). Recent studies have uncovered evidence that differences in internal fat deposition may be responsible (Drackley et al., 2014).

Dairy cattle accumulate relatively more fat in the internal adipose depots (omental, mesenteric, and perirenal) and less subcutaneously compared with

beef cattle. The BCS systems rely mainly on assessment of these subcutaneous fat stores. Nevertheless, in general the correlations among different adipose depots in dairy cows are high, indicating that observed BCS will adequately reflect the non-visible adipose sites and overall body fatness (Roche et al., 2009).

In humans, there is wide variation in the site of fat accumulation, resulting in the so-called "apple" and "pear" shapes. Visceral fat accumulation is linked more strongly with risk for chronic health problems that make up the complex called the "metabolic syndrome". We wondered whether this might be the case in cows during the dry period; might some individuals be more likely to accumulate fat in the internal depots than others, and is internal fat deposition more likely with excessive energy intake (particularly from the starch in corn) during the dry period? Assessment of individual variation is so far impractical due to the lack of economic ways to measure internal fat deposition in cows, but we were able to address the second question in our research.

We randomized non-lactating and non-pregnant cows into two groups with equal starting BCS (Drackley et al., 2014). The groups were fed either a controlled energy, high fibre diet or a higher energy close-up type diet for 8 weeks to mimic a typical dry period. Then, the cows were killed and dissected to determine body composition (Table 1). Surprisingly, despite the huge difference in dietary energy intake the final BCS was not different between groups, although both groups gained BCS during the 8-wk period. However, the masses of internal adipose tissue were greatly increased in cows fed the higher energy diet. Although BCS may provide a very useful indicator of general nutritional adequacy and fat reserves, it may not be sensitive enough to detect potentially important differences in internal fat reserves that develop over the relatively short timeframe of the dry period.

The omental and mesenteric fat depots are located around the digestive tract, and blood that circulates through these tissues drains directly to the liver before reaching the rest of the body. So, large increases in fat mass would mean that more NEFA directly reach the liver during NEB. Furthermore, cytokines and other adipokines produced by adipose tissue also would be increased, which could negatively impact the liver and other tissues. Such changes might help to explain what we have observed in our feeding studies.

We recently completed a second trial with a similar design, except that the controlled energy diet was made even lower in energy density to prevent body weight gain in the non-pregnant cows. Results were very similar, with little difference in BCS but substantial increases in the internal fat depots.

Cardoso et al. (2013) completed a pooled statistical summary of the dry period feeding studies conducted by our group. With over 200 cows per group of controlled energy versus overfed cows, median days to pregnancy was 10

days shorter in cows fed the controlled energy diets. Such a difference might be related to the changes in body fatness even though BCS were not greatly different between groups.

Table 1. Visceral and internal adipose tissues in nonlactating cows fed low energy (LE) or high energy (HE) diets for 8 weeks.

	Diet		
Variable	LE	HE	SEM
Initial BCS	3.00	3.08	0.25
Final BCS	3.55	3.62	0.11
BW, kg	710	722	33
Adipose tissue site			
Omental, kg	17.5	28.1**	1.3
Mesenteric, kg	12.1	22.0**	2.4
Perirenal, kg	6	9.9*	1.2

n = 9 per diet
** $P < 0.01$; * $P < 0.05$ (Drackley et al., 2014)

▪ So What Should *Our* Target BCS Be?

From the standpoint of the cow's biology, the concept of the target BCS argues strongly that a thinner cow (but not undernourished and unhealthy) will be more likely to meet the combined goals of health, production and reproduction. It is to some degree a different question to ask what the optimal BCS at calving should be for best management outcomes.

Until the last decade or so, many experts recommended a higher BCS (3.5 to 4.0) at calving. The rationale was that cows became thin at peak lactation, perhaps having difficulty in conceiving and maintaining a subsequent pregnancy. A higher BCS at calving was thought necessary to provide a "reserve" to let cows "milk off their backs" to avoid this scenario. As we know now, however, striving for a higher BCS at calving actually promotes this scenario rather than preventing it. As Garnsworthy's (2007) research clearly shows, cows with higher BCS lose more BCS after calving. Over time the normal BCS curve (essentially the inverse of the lactation curve) becomes distorted, with higher maximums and lower minimums, all with struggles of transition health problems, poor fertility, disappointing milk yield, and decreased herd life.

The optimal BCS for maximum milk yield may vary across productions systems, as compared by Roche et al. (2009). For example, cows in grazing

systems are more likely to be too thin going into dry-off. Outcomes from differing BCS also are dependent on the genetic potential for milk within those systems. This is shown conceptually in Figure 2. If cows of high genetic merit calve with high BCS they will lose BCS, whereas if they calve in thin BCS they will maintain BCS. In contrast, low-merit cows that calve with high BCS will maintain BCS, but low-merit cows calving in thin condition will gain BCS. All of these outcomes can be predicted from the concept that increasing genetic merit for milk also means that we are selecting for a thinner cow with a lower target BCS. Garnsworthy (2007) estimated that the target BCS for high-merit Holsteins in the UK had decreased from about 2.49 to 2.10 in approximately 20 years. A calving BCS of approximately one-half score unit above the target seems reasonable, which means that BCS at calving should be around 2.75.
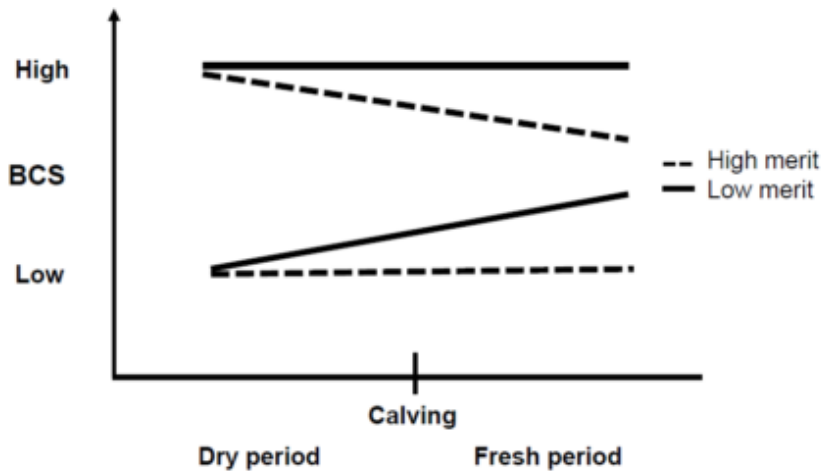
Figure 2. Conceptual depiction of the effect of high or low BCS at calving on BCS change during early lactation in cows of high or low genetic merit for milk production. Based on studies by Garnsworthy (2007) and McNamara (1991).

The concepts demonstrated so eloquently by Garnsworthy's research can be seen in modern large-scale production systems. Carvalho et al. (2014) studied 2 large commercial dairy herds in Wisconsin with the same owners and general management. As shown in Table 2, responses to timed-AI protocols were affected by the BCS change from calving to 21 days in milk.

Pregnancy percentage at either 40 days or 70 days of lactation was markedly greater for cows that gained BCS in early lactation than for cows that maintained or lost BCS, with no difference in energy-corrected milk yield.

These findings are not surprising in themselves and are consistent with long-known relationships between BCS status and fertility. What was surprising, however, was that nearly 60% of the cows in the 2 herds either maintained or gained BCS early postpartum. This evidence contradicts the long-held dogma that nearly all cows are in NEB after calving such that they lose BCS. Cows were thinner on average at calving (BCS = 2.9) than many experts' recommendations. Of interest is that both of these herds used a controlled-energy dry cow program, with a management aim to minimize change in BCS during the dry period and minimize health problems after calving. Anecdotal evidence from many consultants working with high-producing dairy herds in the US confirms that in well-managed herds it is not inevitable that fresh cows must lose BCS.

Table 2. Reproductive and productive responses of Holstein cows (n = 1,887) in two commercial herds in Wisconsin that lost, maintained or gained BCS from calving to 3 wk postpartum.

| | BCS change category | | | |
Item	Lost	Maintained	Gained	*P*
% of cows	41.8	35.8	22.4	
Pregnant to AI at 40 d (%)	25.1	38.2	83.5	< 0.01
Pregnant to AI at 70 d (%)	22.8	36.0	78.3	< 0.01
Pregnancy loss (%)	9.1	5.8	6.2	0.34
BCS at calving	2.93	2.89	2.85	< 0.01
BCS at 21 DIM	2.64	2.89	3.10	< 0.01
Energy-corrected milk[a] (kg/d)	30.9	31.5	28.7	0.30

[a] Mean from calving to d 21 postcalving
From Carvalho et al., 2014

▪ Conclusions

Use of BCS to monitor body energy reserves across the lactation cycle remains a valuable tool for dairy producers and their advisors. Cows have a target BCS that they will attempt to reach, all other things being equal. This target BCS has decreased with time and genetic selection for high milk yield, and likely now is in the range of 2.0–2.5 depending on genetic merit for milk yield. If cows calve with BCS considerably greater than that, they will lose BCS during early lactation and be in substantial negative energy balance. Loss of BCS is associated with greater risk for metabolic and infectious health problems, as well as reduced fertility. Consideration of what makes an optimal BCS score at calving must factor in the welfare, fertility and production implications. Although it may appear a paradox to many producers (and perhaps consumers), healthy cows with relatively thin BCS may have improved welfare and longer productive lives than heavier cows. For most North American Holstein cows, BCS at calving should not be greater than 3.0.

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Influence of Dietary Protein and Amino Acids on Reproduction in Dairy Cows

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■ Take Home Messages

▸ The amino acids that make up dietary protein are important because they provide the building blocks for synthesis of proteins by the cow AND because specific amino acids are used to synthesize other molecules important for biological function.

▸ Overfeeding of protein can reduce energy availability and reduce dairy cow fertility. Lactating cow diets should contain less than 19% crude protein with ruminally degradable protein no more than 10%.

▸ There is less risk to reproduction of feeding diets low in protein although milk yield could be reduced.

▸ New products are being developed to increase delivery of specific amino acids to cows by bypassing utilization by microbes in the rumen. Rumen-protected methionine has been reported to increase lactation performance and reproductive function. Further studies are warranted.

■ Protein – Can't Live Without Them

Protein is a class of nutrient consisting of individual protein molecules, each of which is composed of specific chains of nitrogen-containing amino acids. As a nutrient, protein provides amino acids for the animal to build its own proteins. Each particular protein has a unique sequence of amino acids. The stringing together of amino acids in the right sequence to produce a specific protein is directed by individual genes in the nucleus of the cell. Proteins play roles in every biological process — they are enzymes, (for example, thrombin involved in blood clotting), hormones (follicle stimulating hormone and somatotropin), the major structural component of muscle (actin and myosin) and are secreted into milk to feed the offspring (caseins, lactalbumin).

However, as shown in Figure 1, amino acids are used by animals for more than protein synthesis. Amino acids can be burned as fuel when other energy substrates are limiting. This is one reason why cows in negative energy balance experience weight loss; both fat and protein are mobilized to provide energy.

Besides being used for protein synthesis, specific amino acids are used to make other molecules that have their own biological function. Arginine, for example, is used to synthesize nitric oxide, which, among other things, plays a role in regulating blood flow to tissues. Methionine and lysine are used to synthesize L-carnitine, which functions to utilize fatty acids for energy production in the mitochondria of the cell. Another role of methionine is to participate in export of fat molecules from the liver to prevent fatty liver.

Methionine also plays an important role in the process of DNA methylation. Methylation of DNA can silence specific genes for a short period of time or permanently. One reason cells in the liver don't produce milk proteins, for example, is because the genes for the proteins are shut off by DNA methylation. Feeding rumen-protected methionine has been shown to alter DNA methylation in the early embryo (Peñagaricano et al., 2013). It may be possible, therefore, to regulate specific physiological processes by using amino acids to regulate DNA methylation.
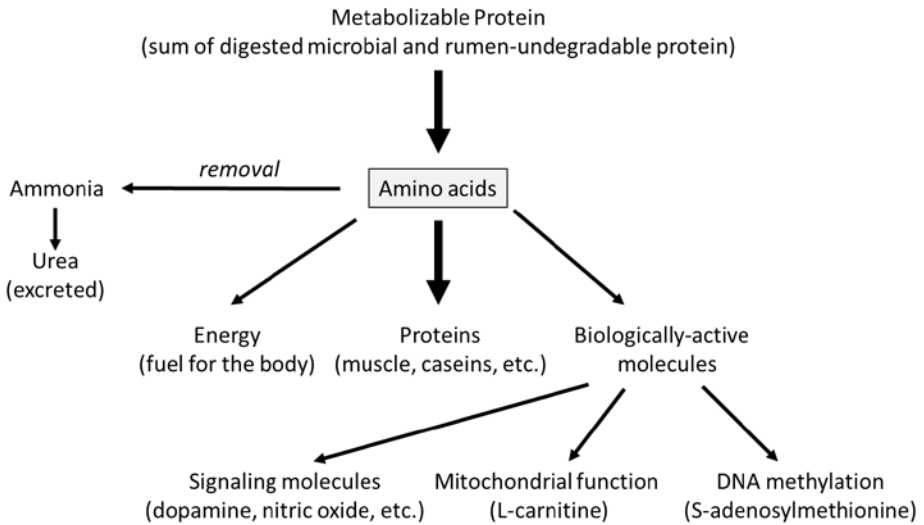

Figure 1. Source and uses of amino acids in the dairy cow.

Animals can synthesize amino acids from other nutrients. Of the 20 amino acids used to make proteins, only 10 of these can be synthesized in sufficient quantity to meet the animal's demands for amino acids. The other 10, which are termed essential amino acids, must be obtained from the diet. The essential amino acids are arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. The most limiting in dairy cattle are lysine, methionine and histidine.

In dairy cattle and other ruminants, there are two main sources of amino acids. Much of the protein in the diet is used by the microbes in the rumen for synthesis of their own proteins. When the microbes pass to the small intestine, microbial proteins are broken down and are used by the cow. This fraction of dietary protein is called microbial protein. The dietary protein not used by rumen microbes is available for digestion in the small intestine and is called rumen undegradable protein. The percent of dietary protein that is not degraded in the rumen can vary greatly depending on the feedstuff and ranges from 0-80%.

Urea, which is derived from ammonia, is also used as a dietary ingredient to increase amino acid availability. While not a protein, rumen microbes can utilize nitrogen from urea for synthesis of amino acids by rumen microbes. Urea contributes to the estimate of crude protein (CP) because CP is calculated based on the amount of nitrogen in the diet. Urea is not effective in young calves (less than 3 months old) because the rumen is not fully functional.

Most, but not all, of the microbial protein and rumen undegradable protein that passes into the small intestine is digested and absorbed into the blood as amino acids. Termed metabolizable protein, this represents the amino acid supply available to the cow for its needs (Figure 1).

▪ Too Much of a Good Thing Can be Bad

The nitrogen in amino acids is converted to ammonia during amino acid degradation. Ammonia is toxic to mammalian cells so it is removed from the cow by conversion to urea and excretion into the urine (Figure 1). Synthesis of urea requires energy so feeding protein in amounts higher than required by the cow can waste otherwise-needed energy. Additionally, urea itself can compromise reproductive function. Feeding high amounts of protein can reduce uterine pH (Elrod and Butler, 1993) and compromise the function of the oocyte or embryo (Rhoads et al., 2006). Given consequences of excess protein for energy metabolism and the function of the oocyte, embryo and uterus, feeding high protein diets have been reported to delay the resumption of estrous cycles after calving, reduce fertility and increase days from calving to conception (Lean et al., 2012; Tamminga, 2006).

Example of a typical experiment to demonstrate the negative effects of overfeeding of protein are shown in Table 1. Cows in this experiment were maintained on ryegrass pastures and began receiving various supplements at an average of 42 days in milk. Diet 1 had the highest estimated CP content (22.8%). Diets 2 and 3 had similar estimated CP (18.0%) but more of the protein was not degraded in the rumen for diet 3 than diet 2. Cows fed the diet with 22.8% CP experienced a delay in first breeding ($P \leq 0.05$) and fewer cows conceived to that breeding ($P \leq 0.05$) than cows fed the 18.0% CP diets. Moreover, there was a tendency for the total number of days non-pregnant to be longer for the cows fed the high CP diet ($P \leq 0.10$). There were no differences in any of these variables between the two 18% CP diets.

Table 1. Effect of protein feeding on reproductive function of lactating cows on ryegrass pastures and fed various supplements in Louisiana, USA (McCormick et al., 1999).

	Diet 1	Diet 2	Diet 3
Estimated crude protein (%)	22.8	18.0	18.0
Estimated rumen undegradable protein (%)	6.4	5.7	8.5
Number of cows	58	61	62
Days to first breeding	90	81	79
Percent pregnant to first service (%)	24	41	39
Days non-pregnant	129	114	114

As a practical matter, effects of excess protein on fertility can be limited by formulating diets so that CP is less than 19% and ruminal degradable protein is no more than 10% (Tamminga, 2006). It is often recommended to monitor urea concentrations in milk or blood to assess protein status. However, the actual correlation between urea concentrations and fertility can be low (reviewed by Sinclair et al., 2014). In a study using records from over 19,000 cows in Poland, the correlation between milk urea concentration and calving interval was significant but only 0.05 (Sawa et al., 2011). It is possible that errors in accurately estimating overall circulating urea status limits the precision of the relationship between urea concentrations in blood or milk and fertility.

▪ Slight Underfeeding of Protein Does Not Seem to Impair Reproductive Function

In several countries, there has been interest in reducing amounts of dietary protein in dairy cow rations so as to reduce feed costs and the discharge of nitrogen excreted by cows into the environment. Sinclair et al. (2014) recently evaluated results of 6 studies to determine whether cows fed diets low in CP experienced reductions in milk yield or reproductive function. Overall, there was a consistent reduction in milk yield for cows in the low CP group (range

12.7–14.5% CP) as compared to cows fed high CP diets (range 16.9–20.0%). The average reduction in milk yield in the low CP group was 1.2 kg/day. In contrast, there was no consistent effect on reproductive function (Figure 2). Thus, it is likely that while feeding too much protein can impair fertility, there is less concern about inadequate protein in the diet, at least with typical dairy cow rations.
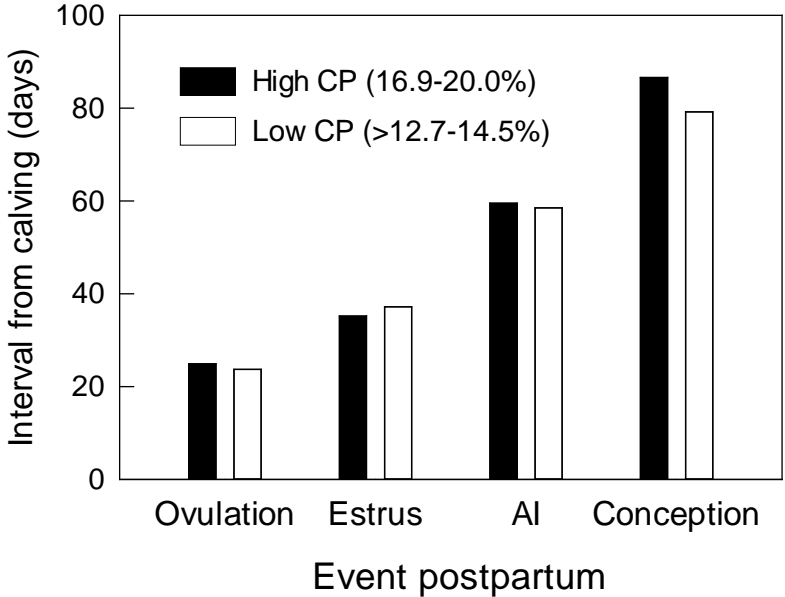

Figure 2. Lack of differences in intervals between calving and ovulation, estrus, artificial insemination (AI), and conception for cows receiving diets higher (high CP) or lower in crude protein (low CP). Sinclair et al. (2014) obtained data from 6 studies in the literature and calculated the average of each interval across studies after adjusting for number of cows in the study.

▪ Prospects for Changing Reproductive Function by Providing Specific Amino Acids in a Rumen-Protected Form

Methionine, which is often the first limiting amino acid in dairy cows, is not only required for milk protein synthesis but also is metabolized into other molecules that play important functions in the animal including those involved in export of lipids from the liver and gene expression (by providing methyl groups used in DNA methylation). A variety of products are available that provide rumen-protected methionine for dairy cattle. Examples include coated

pellets of methionine (for example, Smartamine® M from Adisseo and Mepron® from Evonik) and a chemical precursor of methionine called 2-hydroxy-4-methylthiobutanoic acid (examples are Alimet® and MFP® from Novus and, as an isopropyl ester, MetaSmart® from Adisseo).

Recently, Zanton et al. (2014) performed a meta-analysis on 64 studies that examined effects of feeding rumen-protected methionine. The most consistent effect was increased protein percent and yield. Milk fat percent and yield was increased in some studies. While there was not a significant effect of supplementation on milk yield, there was a trend for a positive effect.

There have been few studies on consequences of feeding rumen-protected methionine for reproductive function. Reliable data on reproduction require larger number of cows that are often used in feeding studies. Effects on reproduction have been evaluated in one study in Iran with Smartamine M (Nikkhah et al., 2013). In that study, which involved 24 animals, cows receiving supplemental methionine experienced, among other things, increases in dry matter intake, milk yield and yield of fat and protein (Table 2). Additionally, there was also improvement in several aspects of reproductive function, including a reduction in days to estrus, AI, and conception. Further research is warranted into whether such positive effects of feeding rumen-protected methionine occur widely.

Table 2. Effect of feeding rumen-protected methionine on function of lactating Holstein cows in Iran during the summer (Nikkhah et al., 2013).

	Treatment	Control	P value
Dry matter intake, kg/day	21.9	19.1	0.01
Milk yield, kg/day	42.4	37.4	0.06
Milk fat yield, kg/day	1.40	1.04	0.002
Milk protein yield, kg/day	1.25	1.02	0.006
Days to first estrus	30.0	52.7	<0.01
Days to first AI	50.5	78.0	0.01
Days to conception	137.0	173.0	0.06
Services per conception	2.8	3.1	0.30

Effectiveness of feeding rumen-protected methionine may vary between products although direct experimental comparisons are lacking. It is also likely that benefits of feeding will be greater for high-yielding cows, cows fed low amounts of metabolizable protein and cows fed diets that are adequate for other important essential amino acids like lysine.

Arginine is another amino acid that can be converted to a variety of biologically-active molecules including nitric oxide and various polyamines. Feeding supplemental arginine in pregnant pigs has been reported to

increase placental weight, litter size and litter birth weight (Bazer et al., 2014; Chacher et al., 2013). Intravenous administration of arginine increased fetal survival and growth in sheep and milk yield in dairy cattle (Chacher et al., 2013). It is possible to increase concentrations of arginine in plasma of dairy cows by feeding N-cabamoyl glutamate, a molecule that can provide glutamate for arginine biosynthesis (Chacher et al., 2013). Perhaps there are opportunities for using N-cabomoyl glutamate for improving milk production and reproduction in dairy cattle. One note of caution — arginine can also adversely affect ovulation and secretion of the pregnancy hormone progesterone. When fed at the beginning at estrus in pigs, supplemental arginine reduced ovulation rate, growth of the embryo, and litter size (Bazer et al., 2014). The need for precise timing of administration of supplemental arginine may limit its effectiveness in dairy cattle systems.

▪ Conflict of Interest

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Align Your Precision Dairy Robot System with Your Goals

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▪ Take Home Messages

‣ The value of precision technology increases if all sensors are used in a single information system using the aggregated information. It is not about management by exception anymore, but about valuating fitness scores of the healthy cows.

‣ Implement precision information in each step of your management circle: 1) vision, 2) design, 3) goal, 4) observe, 5) analyze, 6) adjust, 7) evaluate … and back to step 3), to make the management circle go round based on the best possible information.

‣ Include precision information in short term and long term management sessions with your dairy advisory team. Require from your advisors a scenario (based on precision settings), rather than a single step advice, to be able to steer the process in between the consultancy visits.

‣ Free cow traffic in robot systems give about 1 kg of milk/cow/day more, or 76 kg milk/robot/day more than guided/forced cow traffic. Cow pens with 1 milk robot per pen give 60 kg milk/robot/day less than in pens with more than 1 robot.

▪ Introduction

Many studies, publications and practical testimonials are available that show the merit and values of precision dairy sensor technologies. Usually these publications deal with 1 sensor at the time only and mostly show the value on the optimistic side, so that companies can get them sold.

Great work is done by Jeffrey Bewley, UKY to summarize the value of these individual monitoring tools available. He concludes that there is a gap between the impact of precision dairy farming technologies in research versus commercial settings. Additional effort needs to be directed toward

implementation of management practices to fully use information provided by these technologies. Factors that have the most influence on the profitability are those related to what happens with the technology after it is purchased (Bewley, 2013).

In addition, multiple sensors in combination with the visual observation of naturally behaving cows in the robot barn enable the herdsman to see the abnormal individual in a much earlier stage. It is about being pro-active instead of reactive and about making better decisions to prevent cows drifting off (instead of making culling decisions after the problem got too severe). All of this improves constant health and therefore constant production, fertility and longevity results of the herd (Smink, 2012).

Precision dairy does not change cows or people, but it will change how they work together. The path to success using precision tools on farm is to maintain realistic expectations, support the farmer in using the information, never lose sight of the cow and educate, communicate and collaborate (Bewley, 2015).

In this paper I would like to make the next step and show:

- how dairy producers can utilize precision technology wisely in daily practice;
- how dairy producers can get trained to get value out of the sensors;
- how dairy consultants can use sensors to provide complete up to date advice.

▪ Circle of Efficiency and Management

To structure the practical use of precision technologies, I will use an adjusted circle of efficiency and management (Figure 1) and will go over each step of this process and conclude how this circle can go round with precision information in the collaboration between cow, herdsman and consultant.
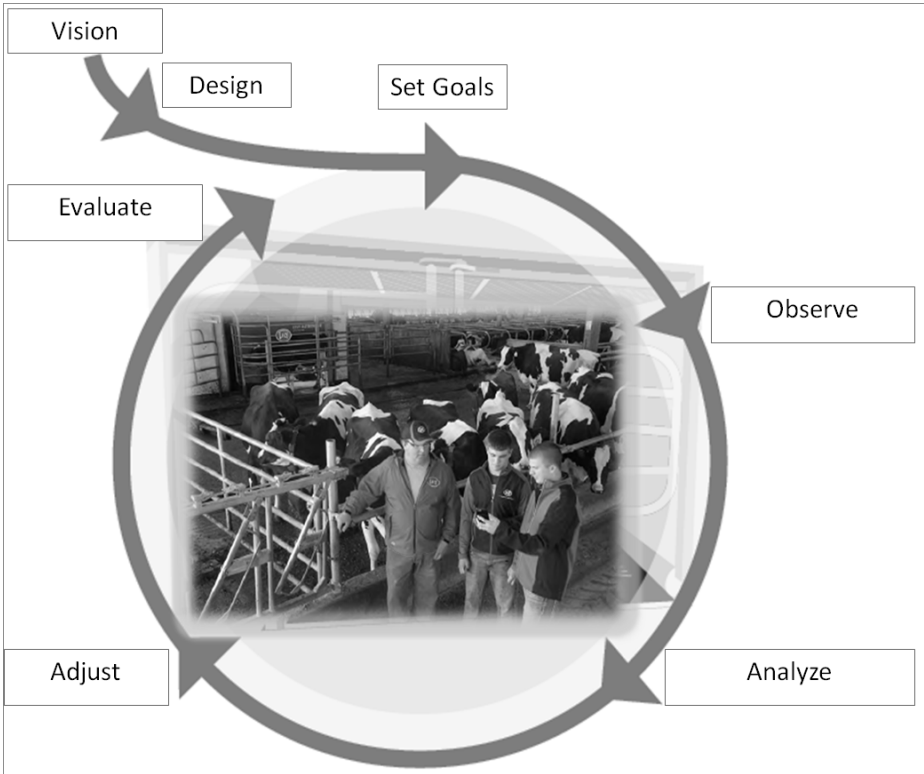
Figure 1. Precision dairy circle of efficiency and management.

Step 1: Vision

Without vision there is no way to go. Every producer will have a vision of where he wants to be with his farm in a decade or two. All goals, activities and choices made will have to point in that direction. It is important for the consultant to know and understand what the vision of the different generations of the farm are to be able to perfect a suitable advice stream.

Globally, we will have to feed over 9 billion people in 2050 according to the FAO (Food and Agricultural Organization of the United Nations). So, as a dairy industry we will have to be very thoughtful of our resources, such as land, feed, water, and will have to prevent pollution and limit waste. However, the key resource on a dairy farm is the herd of cows; therefore, as an industry we need to optimize each individual cow, which also is the precious beloved resource in the perception of the consumer. What is good for the cow and her health is also good for her talents to produce milk efficiently. Both aspects are of high interest for consumers.

What does this mean for cow management in practice: 1) Get the best out of the cow and let her show her unused production talents. 2) Observe and secure her health to tune her capabilities for tomorrow. 3) Match genetic potential of ancestors to create a better offspring. 4) Use the benefit of genetic evolution, but be cautious about what the data really tell you, especially if they are based on general monthly information or once in a lifetime measurements used by breeding associations.

Precision monitoring allows for precision handling as well; for example, no waste of the resource feed, by feeding cows according to their general needs in the bunk combined with individual portions according to their individual potential in the station or robot, with a focus on Income Over Feed Cost (IOFC) to make it financially sound.

Step 2: Design Fundament to Your Farm

Only once in a generation a producer really has an opportunity to design his barn. Every single choice will have an effect on cow comfort and her talent to efficiently use her resources. Four important factors to consider when designing a robot barn:

1) Free or Guided Cow Traffic in the Robot Barn

Analyzing data from 635 North American dairy farms with automated milking systems (AMS) for risk factors associated with increased milk production showed that free cow traffic was associated with increased production per cow/day and robot/day compared to guided systems. Free cow traffic was associated with 1.1 kg more milk/cow/day and 67.2 kg more milk/robot/day than guided cow traffic (Tremblay et al., 2015).

Let's put some perspective on these numbers: if there are approximately 20,000 Lely milk robots in the world with free cow traffic, which each producing an additional 67 kg milk per day, that would equal to an additional 40 truck loads of milk per day available to the global population.

2) Cow Pen Size with One, Two or Three Robots

The same analysis with the 635 North American AMS farms showed that a single robot cow pen was associated with decreased production/robot/day compared to pens with 2 or 3 robots per pen. On average one robot cow pens produced 59.8 kg less milk per robot/day than 2 or three robot cow pens (Tremblay et al., 2015). The production difference between robots per pen becomes larger as milkings decrease when the barn gets filled to its capacity (Tremblay et al., 2015).

3) *Efficiency Based on Cow Comfort and Cow Touches*

Every living creature is most efficient if it is comfortable and gets what it needs 24/7. Cows also need a comfortable life without losing energy on the wrong activities like waiting in line for feed, water or milking. This means that interruptions in the cow's day to day life have to be limited to a minimum by designing a barn where the general population is not disturbed when 'human touches' to other cows take place. The good news is that precision dairy technology also secures efficient cow touches on an 'as needed' base only, leaving the rest of the herd alone to increase efficiency of labor in the barn.

4) *Precision Feeding*

Feed is the main variable cost on the farm, which needs to be well managed to get the desired IOFC. Most of our current feed principles are developed a few decades ago based on circumstances without precision tools or information. Lely International found an increase of 1.9 kg milk/cow/day with robotic TMR feeders as a result of higher dry matter intake and continuous fresh feed at the feed bunk 24/7 (web site lely.com - farming tips). When designing a new facility make sure it allows for future implementation of these automatic feeding technologies.

Step 3: Set Goals

After vision and design are set, it is time to define both long and short term goals. The long term goal will determine which precision dairy technology tools could provide added value to the farm processes. The short term goals will determine which alerts and information are needed to optimize the use of the resource 'cow' on the farm.

Make these precision dairy goals SMART (specific, measurable, achievable, realistic, and time-bound) and use them to find the critical success factors on the farm to achieve these goals. For example: longevity, fat/protein per day, occurrence of abdominal or udder health problems.

Based on these factors you make a dashboard with your precision information, which can be used on a daily basis to observe the results and see whether you are 'driving in the right direction' and 'staying on track'. The producer's job is to manage the daily monitoring and to align barn workers with proper observation and work routines. However, it is the consultant's job to provide the right training and support to read these dashboard tools and provide scenarios for the herd manager to steer his results on a daily basis, rather than monthly 'after the fact go, no-go feedback' kind of advisory steps.

Step 4: Observe

The better robot systems will measure more than 120 values per cow per day. All those data points by themselves are useless and have to become meaningful for the producer and his consultant. Meaningful data lead to action. Every single value or attention a herd manager gets presented with has to lead to either actions to help cows reach the goals that the producer has set, or to save resources or time for cows/people to stimulate the goals.

Steeneveld and Hogeveen (2015) found a disconnect between the economic theory not matching the reality. Sensor systems were associated with a higher average production per cow on AMS farms and with a lower average production per cow on conventional farms after investment.

A pro-active attitude to use AMS sensor data pays off in lower somatic cell counts (SCC). A passive approach (wait till individual SCC increases, clots on filter) to decide whether a cow needs treatment will result in a higher SCC (Tol van der, 2012). The combination of milk conductivity, milk color, pre-milk time and yield per quarter lead to an action list for clinical mastitis prevention. Another example is if we see high fat/protein ratio in combination with rumination drifting off, the program indicates ketosis. Similarly, if a cow shows normal milk production, normal conductivity and color of the milk in combination with high milk temperature, we know the cow is sick but not very likely caused by mastitis.

It is the quality of the individual sensors, together with the right dashboard of meaningful summarized information along with the analysis capability of the cow person, to combine the digital picture of the data with the physical observations in the barn. We need good cow people to combine the cow signals with the summarized action data from sensors and find the cow before she has a problem!

For the producer this means:

1. Select only meaningful performance indicators to use every day.

2. Develop a solid routine to read these and change them into actions.

3. Tune the parameters so that an attention list becomes an action list for every worker in the barn.

4. Track what you treat and match weekly what the difference is between action and treatment to fine tune your action list depending on the experiences collected.

5. Require training and support from your consultant/provider to maintain proper settings.

6. Be critical of the supplier's system defaults and make sure parameters are set depending on <u>your</u> goal and not the goal of the supplier.

7. Use smart solutions like InHerd mobile apps to have the digital picture of the cow next to the physical picture you observe while touching the cow in the barn for an efficient work stream and to get most out of the info available.

Step 5: Analyze

On a regular basis the producer has to bring all knowledge together in a dairy advisory management team. Successful teams have helped dairy businesses to improve milk yield and quality, efficiency of workforce and IOFC or return on assets. Advisory teams can consist of veterinarian, nutritionist, robot expert, accountant, lender and extension educator who work with you on a regular basis; the teams may also include non-farm or non-agricultural members as well as other dairy producers (Holden, 2014). This advisory team analyzes results, measures the progress made and determines the most important gaps to come to a series of scenarios to be used in the following month.

All summarized sensor data graphs bring facts to these advisory gatherings. The following aspects need to be considered:

1. last month's progress as set in the dashboard defined in step 3 (Set Goals)

2. daily progress regarding milk yield, robot visits, feed intake, fat and protein levels, body weight and rumination

3. lactation progress (separate for first calf heifers and mature cows) regarding milk yield, robot visits, feed intake, fat and protein levels, fat and protein ratio and body weight

4. current lactation/yield distribution to fine tune feed bunk rations and set the sweet spot between the attraction of cows to the robot and the money spent on the ration at the feed bunk and on the robot

5. udder health status looking at progress of both current cases and new cases, using combined udder health indicators (conductivity, color, pre-milk time, quarter yield contribution, SCC, yield deviation)

6. body health status using the combination of rumination, body weight deviation, fat/protein ratios, milk temperature, yield deviation and feed intake

7. robot performance and usage: determine cow robot efficiency and check whether every milking is a useful milking and whether every minute a robot spends on a cow is adding value to milk in the tank

In general, the focus has to be on finding wastes of all possible resources (including the resource cow and robot time) and finding unused cow production talents.

For the advisor this means that you'll have to change your mind set and probably have to learn a few new things. The famer gets wiser with so much more information on a daily basis, and he will heavily lean on your professional expertise to generate scenarios for the coming period based on the findings and gaps measured each time you meet.

Step 6: Adjust

Based on the gaps found in the analysis, plans and settings can be adjusted. Thinking of precision dairy tools this means that you, the producer, should:

1) stick with your goals and choose the adjustments that bring the best result to your short term goals

2) only change 1 factor at a time, so that you always know what the effect is. If you change 2 factors with opposite results at the same time you will not see progress, although 1 of the 2 could potentially be the key factor to progress…..

3) adjust the dashboard parameters accordingly, so that the action lists presented by the system bring focus to the short term goals at hand.

Step 7: Evaluate

This step should be a twice per year reflection on:

1) the achievements in the past 12 months

2) renewed assessment of the critical success factors in the coming year

3) adjustment of the short term goals

4) adjustment of the precision information dashboard and action plans

As a producer be critical on your advisors. The evaluation assessment is there to show whether they were right or not in their scenarios and whether they will have to learn or adjust as well.

As an advisor be critical on your producers. The evaluation assessment is there to show whether they were right or not in the follow-up of your scenarios and sticking to action plans on a daily basis. One of your tasks is to hold producers (and their workers) accountable for their job in a positive way.

The evaluation step also requires benchmarking with other producers who are in the same circumstances. Historically, the DHI data have been used for very practical and good reasons. They are mostly good to use, as long as you are able to compare apples to apples.

Now with precision technology many producers ask themselves whether to stay connected with the DHI and the answer is YES for two reasons: 1) DHI makes it possible to collect data for the genetic advancements of our industry. If you quit DHI then do not ask your breeding association or semen suppliers to give you advice on bull selections; 2) DHI offers lots of opportunities for cultures and tests not available in sensors (yet) and they could be very useful for problem situations.

Many alternative benchmarking tools are available, which are based on all the precision information available on the farm. For example, Lely robot users have an integrated benchmark application where producers can compare information with each other based on all robot sensor data. A tool like this is also available as a smartphone app called FarmVisit, which can be used by the advisors too (website: lelyt4c.com).

Precision technology and the connection of all these systems is also an opportunity to make the next step in benchmarking. All herds' production data and management information can be clustered in a meaningful way using cluster analysis. This clustering approach will yield improved peer groups of farms compared with benchmarking methods based on criteria such as country, region, breed, or breed and region.

Tremblay et al. (2015) applied mixed latent-class model-based cluster analysis to 529 North American AMS dairy farms with respect to 18 significant risk factors and defined 6 meaningful clusters. Each cluster (i.e. peer group) represented unique management styles, challenges and production patterns. When compared with peer groups based on criteria similar to the conventional benchmarking standards, the 6 clusters better predicted milk produced per robot per day. Each cluster represented a unique management and production pattern that requires specialized advice. For example, cluster 1 farms are farms that recently installed AMS robots while cluster 3 farms, the most northern farms, feed high amounts of concentrates through the robot to compensate for low energy feed in the bunk. In addition to general recommendations for the farms within a cluster, individual farms can generate their own specific goals by comparing themselves to farms within their cluster based on percentile ranks. This is very comparable to benchmarking, but

adds the specific characteristics of the peer group resulting in better farm management advice. The improvement that cluster analysis allows for is characterized by the multivariable approach and the fact that comparisons between production units can be accomplished within a cluster and between clusters as a choice (Tremblay, 2015).

And Then Fine Tuning: Let the Circle Go Round

This is a key phase in the whole process, because here the circle is completed and if this is not done right the wheel will not start to turn for continuous improvement. The key elements to make this circle a perfect running wheel are as follows:

1. Connect every evaluation with a new starting point to set new short term goals and further tuning of precision tools.

2. Stick to your plans and keep all team members and advisors accountable for their contribution.

3. Don't be afraid, but keep an open mind for different ways of thinking.

4. Use the best suitable benchmark for the goals of the farm.

Keep an eye for the global challenges we have as an industry: feed over 9 billion people in 2050. As a dairy industry we will have to be very thoughtful of our resources — land, feed, and water — and prevent pollution and limit waste, not only on a large scale, but also on a farm and an individual cow scale.

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ADVANCING ANIMAL AND HUMAN HEALTH
WITH SCIENCE AND COMPASSION

What's new about Digital Dermatitis?

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ADVANCING ANIMAL AND HUMAN HEALTH
WITH SCIENCE AND COMPASSION

March 10th, 2016

Introduction

- Problem Farm -> Manageable State of DD

- Aids for
- DD Prevention and Control,
- Recognition of Trends
- Topical Treatment, Hoof baths
- Chronic Consequences of DD

- More Research and Information …

…less, applied earlier …is more!!!

Prognosis DD

…not all M2 are equal!

Different Prognosis depending on Cow Type…
CHRONIC consequences of DD

New Aids for recognizing DD Trends

- Cattle Lameness Book (with e-book)
- DD Check App
- (iOS, Trends, Tx, Prediction model)

…Breaking the DD Cycle

hyperkeratotic   proliferative

M0   M1   M4.1   M2   M4   M3

<2cm

>2cm

Berry 2012, Döpfer 1997

Topical Treatment of DD...

- Recurrent cases of DD react differently to topical treatment! Recognize early, treat promptly!

1-2 days post tx:
M2 -> M3, the reservoir remains!

Focus DD – Wraps – "Bikini wrap"

- To wrap or not to wrap?

...less,
applied earlier
is more...

The VFD will become mandatory in 2017

- Consequently:
- the use of antimicrobials such as tetracyclines for the
- topical treatment of DD lesions will have to be
- applied within a valid veterinarian-client relationship
- No over-the-counter antimicrobials used for human treatments will be available anymore.
- NEED for non-antimicrobial prevention and control of DD!!!

3 Key Messages

- When you lift a foot to treat DD, you are too late!
  - treponemes penetrate deep into the epidermis and dermis and DD has long-term consequences
- The dynamics of DD are driven by chronic DD lesions,
  - not by active DD lesions (M2) alone
- Need for long-term
Integrated Prevention and Control of DD
  - from calves -> heifers -> adult cows/steers -> dry cows
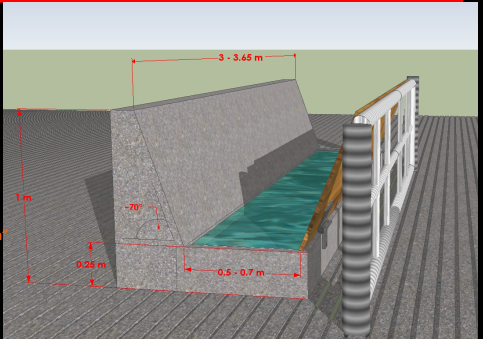
"Manageable State of Disease"

Customized Prevention and Control...

First Sign of succes is:

Less Proliferative DD !!!

Dimension – Design – Behavior

The "Ideal Hoofbath" Cook et al 2012
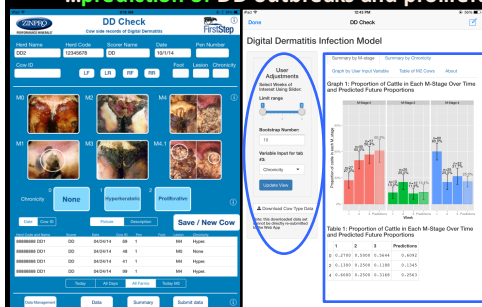
Cow passages

- Systematic **Footbath sampling and cultures** reveal

- **Exponential increase** of aerobic and anaerobic bacteria after 150 - 300 – 350 cow passages

- depending on leg **hygiene**

- **Customized advice about footbath management**
- **Less, applied earlier is more!**

DD Breaking the Cycle…

- **DD Check App….free** from AppStore
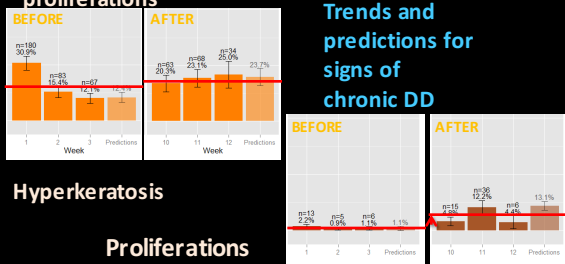- **…prediction of** DD outbreaks and proliferative trends

Trends

Prognose for treatment of M2

Collect, analyze, share data…

DD Breaking the Cycle

- **DD Check App….free** from AppStore
- **…predictions** of DD outbreaks and trends in proliferations

Trends and predictions for signs of chronic DD

Hyperkeratosis

Proliferations
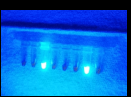
Chronic DD is for Life!

- **Chronic Sequelae** of DD in seven groups
- DD is for "Life"!

- **We have not even begun to estimate the true losses caused by DD**

There is more…

- **ELISA** tests for treponemes subtypes
- **PCR, LAMP detection of** treponeme subtypes
- **MIC/MBC lab testing of chemicals**
- **SNP genotyping** for dairy and beef ->
  - pre-screening of cattle
- **Microbiomes** of DD lesions (Zinicola et al 2015)
- **Microbiomes** of feces under treatment and control

- **Agent-based pathogenesis modeling**

More research and infos

- **Webinars, Press releases – beef and dairy**
- **Folder, posters**
- **App, App…Models**
- **Books…."Cattle Lameness"**
- **ICAR claw health atlas:**
www.icar.org/Documents/ICAR_Claw_Health_Atlas.pdf

- **Workshops**

- **dopfer@wisc.edu FAPM website,**
- **UW-Madison, Wisconsin, USA**

Have you hugged your epidemiologist today?

Is Automated Calf Feeding Right For Your Farm?

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▪ Take Home Messages

▸ The percent of dairy operations housing preweaned calves in groups has increased in recent years in the U.S.

▸ Computerized, automated calf feeders make it easier to feed young calves in groups.

▸ Larger amounts of milk and more frequent meals can be delivered with an automated feeder without additional labor required.

▸ Housing young calves in groups can increase incidence of disease.

▸ Information on feeding behaviour provided by the feeder software can help identify sick calves. Human observation is also critical.

▸ Management practices such as cleanliness of equipment and housing, high quality milk, small group size, good ventilation and adequate feeding regime are important for successful use of automated feeders.

▸ It takes excellent management for the system to work. Installing a feeder and not spending the time and effort to make it work will result in system failure. Are you committed to making it work?

▪ Housing Calves in Groups

The majority of pre-weaned calves in the U.S. (about 75%, USDA 2007) are housed in individual pens or hutches until after weaning; however, interest in automated calf feeders used to feed calves in groups has been growing in the U.S. Automated calf feeding systems make it more convenient to house calves in groups where the calves can interact with each other and drink milk many times a day without necessarily increasing human labor. There is very limited research in the U.S. on best housing, ventilation and management practices to be used with these automated feeders.

Individual calf housing has advantages for animal welfare, such as the reduced transmission of infectious diseases as a result of limited physical contact between calves. In addition, individually housed calves are easier to observe, which can result in more effective disease treatment. There also is less competition for food between calves with individual housing. However, there are also potential welfare disadvantages with individual housing. The most obvious ones are the lack of social contact among calves and the limitation of movement by the reduced physical space provided. In addition, individually housed calves are usually fed only twice a day.

Automated feeders can provide pre-weaned calves either cow´s milk or milk replacer and water individually in a controlled manner. Calves are housed in a group and identified using radio frequency identification (RFID) tags. A processor integrated into the feeder ensures that the milk quantity is allocated according to prescribed parameters, such as age, and dispensed over several feedings per day. The milk replacer concentration, feed quantity per visit, and total feed allocation per day can automatically adjust to the calves' physiological development or age. Cow´s milk alone or combinations of cow's milk and milk replacer can also be fed, dispensed and adjusted according to a predefined plan. Weaning can be done automatically and gradually according to age or intake of solid food.

Feeding group-housed calves on an automated milk feeding system was shown to require less labor time than when calves were housed individually, helping offset the initial investment cost of the machines (Kung, 1997). This might not be the case on every farm, as in order to use the system successfully, a similar amount of labor time might still be required. Based on our survey, the expectation of reduced labor is one of the main reasons why producers invested in automated feeders.

Dairy producers might be interested in purchasing automated calf feeders partly because of labor savings, but the ability to feed calves many times a day, a more natural behavior, is also an advantage. Our research team has collected data from many operations using automated feeders to document labor costs. It appears that labor time is not necessarily reduced, but the type of labor changes. Calves still need to be observed, pens cleaned, equipment cleaned and sanitized, etc. However, it would be very labor intensive to feed calves 4 to 6 times a day without automation.

An advantage of using the automated system compared to manually feeding calves twice a day is that the feeders allow for distribution of the total daily milk intake into small meals throughout the day, with no extra labor input, allowing a greater amount of milk to be fed without requiring the calf to drink a very large amount at each meal. These automated systems also can monitor the feeding behavior of each calf, such as number and timing of visits, the amount of milk consumed by each calf, and the number of unrewarded visits

(when no milk is fed), which has been shown in controlled research studies to help identify sick calves (Borderas et al., 2009).

Efficiency of automated feeders can be improved if the amount of time that each calf spends at the feeder in visits when it is not entitled to be fed is reduced. Feeding larger amounts of milk reduces the number of these unrewarded visits. In addition, automated feeding systems need to be managed properly to avoid competition. Potential strategies would be to keep group sizes relatively small, to properly introduce new calves to the group with adequate training, and to feed higher quantities of milk and in larger meals (4 times a day instead of 8 times a day). Many of these points were well addressed at this conference last year on a review by Steele et al. (2015).

Are any of the above mentioned strategies being successfully used on farms with automated calf feeders in the upper Midwest of the U.S.? There has been consistent growth in the upper Midwest U.S. on the number of farms installing computerized automated calf feeders. No research had been done in our region; therefore, we collected on farm data to learn what strategies are most common in typical Midwest herds. Automated calf feeders represent a new technology that needs study in order to understand housing and management characteristics that enhance calf welfare and dairy operation profitability.

This article summarizes some of the findings of a longitudinal field study we are conducting at the University of Minnesota involving 38 farms with calf feeders. These types of studies can provide descriptive information on housing and management practices, and by collecting many animal and facility measurements, we can identify factors that are associated with successful use of these systems. This methodology does not provide a direct 'cause and effect' connection, but we can identify guidelines and factors that can be important and then adopted by producers or investigated in more detail.

▪ Housing and Management Practices in the Midwest U.S. Automated Calf Feeder Facilities

Our study showed that 61% of the farms retrofitted an older facility (tiestall, pig barn, chicken barn, etc.) into a calf facility whereas the remaining 39% built a new barn specifically for the preweaned calves. We did not find a difference in calf health between new and retrofitted barns. Of these facilities, 50% were naturally ventilated barns, 39% were mechanically ventilated, 8% were additions to tunnel ventilated barns, and 3% were naturally ventilated "igloos." A great majority of facilities (87%) supplemented ventilation systems with positive pressure tubes. It is important that dairy producers work with an experienced engineer when designing a new barn or retrofitting an old one to

make sure all important aspects of ventilation and layout are properly considered.

The average number of calves per pen was 18.2 (Figure 1) which is less than the maximum suggested by the manufacturers (up to 30); the space per calf within the pen was 4.6 sq. meters. There was a wide distribution among farms.
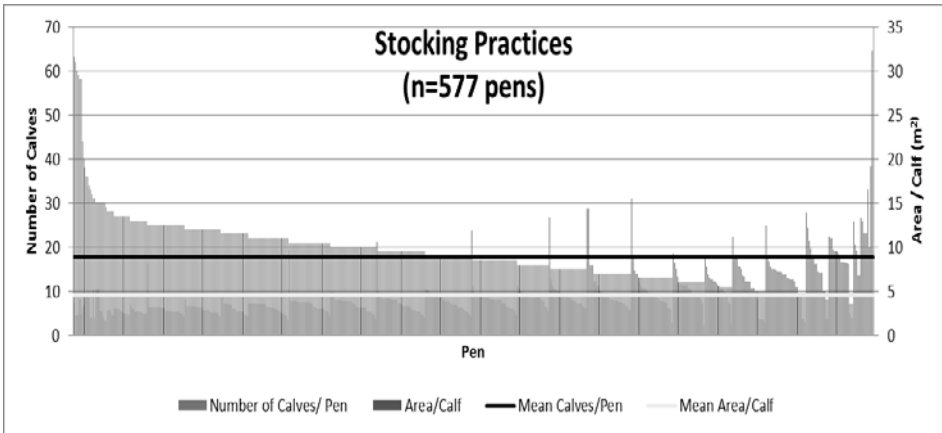

Figure 1. Stocking density as number of calves per pen and area per calf.

Average peak milk allowance was 8.3 liters per day and start milk allowance was 5.4 liters per day (Figure 2). A total of 68% of farms fed calves reconstituted milk replacer, 24% fed whole milk plus replacer or protein balancer, and 8% fed unsupplemented whole milk. Mean time from feeder introduction to peak milk allowance was 18 days.

Figure 2. Starting and peak amounts of milk/milk replacer fed.

Calves were placed on the feeder group at 5.4 days of age (range of 0 to 14 days; Figure 3); 10 farms placed calves in the group at zero or one day of age. Placing calves on the feeder at a younger age requires more training and observation to make sure that calves are able to drink their required amounts of milk.
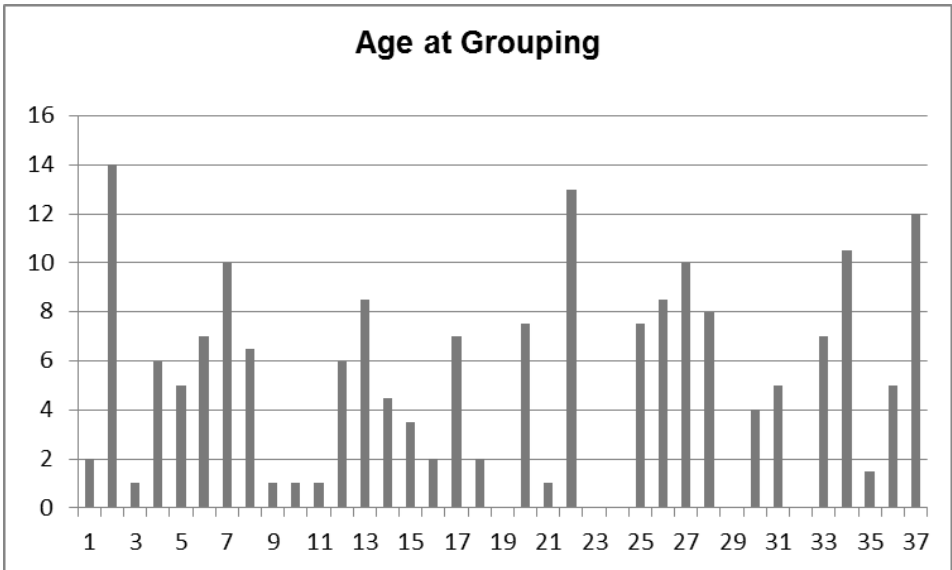

Figure 3. Age of introduction to group pen with automated calf feeder.

▪ Calf Health Observations

During each visit, calves (n=10,179) were scored for health by a single observer using four categories: attitude (0–4), ears (0–4), nose (0–3), eyes (0–3), and cleanliness (an indicator of diarrhea, 0–2), with 0 representing a normal, healthy calf. Body temperature was measured if a calf had an abnormal health score. In addition, blood was drawn from any calves one to five days old (n=985) and serum protein concentration used to assess passive immunity transfer. Milk samples were collected from the mixing container inside the feeder and at the end of the hose (tube) nearest to the nipple for measurement of standard plate count (SPC) and coliform count.

Figure 4 summarizes the calf health scores for the top 10th and the bottom 10th percentile farms. There was considerable variation among farms, indicating that housing and management factors can definitely influence the success of using these feeding systems. Table 1 summarizes the SPC and coliform counts for the top and bottom farms for the samples collected from the mixer and the hose (or tube). Again, there was a lot of variation and some very extreme numbers were detected. The milk the calf is drinking should have less than 100,000 CFU/ml for total plate count.
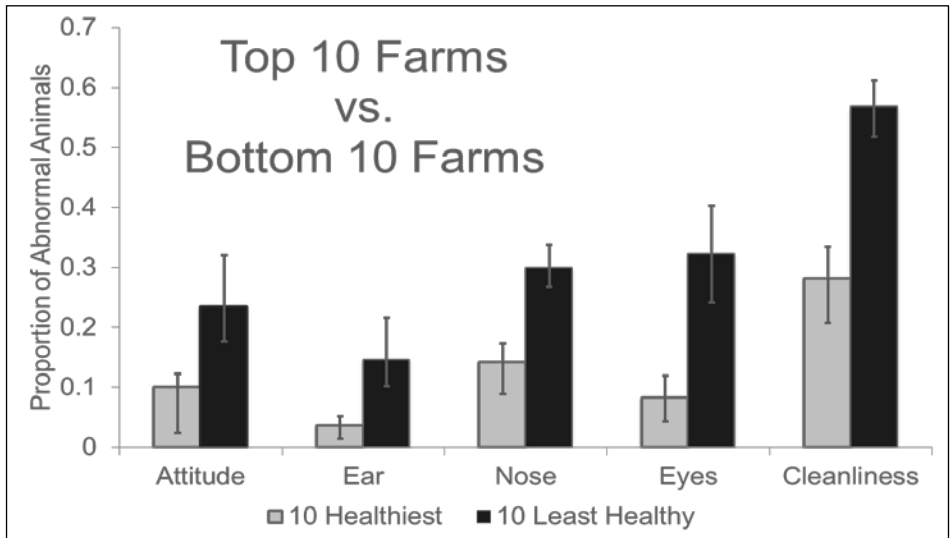

Figure 4. Average proportion of abnormal health scores.

Table 1. Farm average bacterial counts (cfu/ml) across visits for top and bottom 10 farms.

Item	Tube Coliform	Mixer Coliform	Tube SPC	Mixer SPC
Median of Top 10 (Q1-Q3)	887 (206-1,211)	12 (3-15)	87,590 (32,603-134,940)	9,006 (2,308-9,392)
Median of Bottom 10 (Q1-Q3)	5,659,567 (1,198,059- 14,344,063)	522,263 (64,564- 20,001,213)	21,140,625 (18,644,538- 71,642,610)	10,209,920 (3,204,500- 43,673,293)

▪ Risk Factors for Abnormal Health Scores

We conducted a mixed model statistical analysis to investigate the association of various housing and management factors with calf health. The factors listed below were associated with abnormal health scores; therefore, farms that have these characteristics are more likely to have more sick calves and be less successful using an automated calf feeder system.

▸ Number of calves per group: farms with greater numbers of calves per group had a higher number of sick calves.

▸ Space per calf: less space per calf was associated with higher number of abnormal scores. This was independent of group size. What this means is that a small group size with not much space available to move around the pen could still be a problem. This would be an important consideration when determining the pen size.

▸ Time to reach peak milk allowance: farms that waited longer to reach the maximum amount of milk had worse health scores. Most farms increased the amount of milk incrementally rather than offering a large amount of milk from day one. That is a good management practice, but the analysis indicated that it is better to achieve the peak amount in a shorter number of days, for example 8 days instead of 18 days. Plane of nutrition is important.

▸ Air speed in resting area and at the feeder: faster air movement at the resting area was associated with worse nasal scores, an indicator of respiratory disease; air speed at the feeder was associated with abnormal ear scores. This result can be an indication that ventilation is important, but drafts are undesirable.

▸ Standard bacterial plate count (SPC) on hose (tube) milk samples greater than 100,000 cells per ml: higher counts were associated with higher number of calves with abnormal health scores. We need to provide high quality, clean milk to calves.

▪ Why Use an Automated Feeder?

Dairy producers were asked the top reasons for purchasing the automated calf feeder. In order of priority, their top responses included:

1. less time spent on menial tasks
2. improved calf growth rate
3. improved information on calf feeding
4. natural diet changes/ more natural feeding
5. improved labor condition
6. reduced labor cost
7. social interaction between calves
8. ability for calves to express natural behaviors

▪ Conclusions

Automated calf feeders are growing in popularity and this trend will probably continue as producers want more flexible labor management and consumers want animals to have a more natural life. Feeding calves in groups allows calves to express some natural behaviors that cannot be expressed when housed individually, but offers some challenges in relation to maintaining good health, another important aspect of good animal welfare.

It was interesting to learn that producers might not be aware of the need for cleaning the equipment on a routine basis, which resulted in a wide distribution in the cleanliness of the milk that the calves were drinking across farms. It is extremely important to run all the circuit cleaning as recommended by the manufacturer (or more), replace hoses and nipples regularly, use a good disinfectant (such as chlorhexidine) to remove biofilms from the surfaces, keep the area around the feeder clean, provide clean and dry bedding to the calves, have good quality milk, calibrate the equipment to deliver appropriate concentration of nutrients and temperature for the milk, etc.

Good health is certainly achievable when using automated calf feeders to raise preweaned calves as long as appropriate management and maintenance are emphasized and implemented.

Acknowledgments

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Is Automated Calf Feeding Right For Your Farm?

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▪ Take Home Messages

▸ The percent of dairy operations housing preweaned calves in groups has increased in recent years in the U.S.

▸ Computerized, automated calf feeders make it easier to feed young calves in groups.

▸ Larger amounts of milk and more frequent meals can be delivered with an automated feeder without additional labor required.

▸ Housing young calves in groups can increase incidence of disease.

▸ Information on feeding behaviour provided by the feeder software can help identify sick calves. Human observation is also critical.

▸ Management practices such as cleanliness of equipment and housing, high quality milk, small group size, good ventilation and adequate feeding regime are important for successful use of automated feeders.

▸ It takes excellent management for the system to work. Installing a feeder and not spending the time and effort to make it work will result in system failure. Are you committed to making it work?

▪ Housing Calves in Groups

The majority of pre-weaned calves in the U.S. (about 75%, USDA 2007) are housed in individual pens or hutches until after weaning; however, interest in automated calf feeders used to feed calves in groups has been growing in the U.S. Automated calf feeding systems make it more convenient to house calves in groups where the calves can interact with each other and drink milk many times a day without necessarily increasing human labor. There is very limited research in the U.S. on best housing, ventilation and management practices to be used with these automated feeders.

Individual calf housing has advantages for animal welfare, such as the reduced transmission of infectious diseases as a result of limited physical contact between calves. In addition, individually housed calves are easier to observe, which can result in more effective disease treatment. There also is less competition for food between calves with individual housing. However, there are also potential welfare disadvantages with individual housing. The most obvious ones are the lack of social contact among calves and the limitation of movement by the reduced physical space provided. In addition, individually housed calves are usually fed only twice a day.

Automated feeders can provide pre-weaned calves either cow´s milk or milk replacer and water individually in a controlled manner. Calves are housed in a group and identified using radio frequency identification (RFID) tags. A processor integrated into the feeder ensures that the milk quantity is allocated according to prescribed parameters, such as age, and dispensed over several feedings per day. The milk replacer concentration, feed quantity per visit, and total feed allocation per day can automatically adjust to the calves' physiological development or age. Cow´s milk alone or combinations of cow's milk and milk replacer can also be fed, dispensed and adjusted according to a predefined plan. Weaning can be done automatically and gradually according to age or intake of solid food.

Feeding group-housed calves on an automated milk feeding system was shown to require less labor time than when calves were housed individually, helping offset the initial investment cost of the machines (Kung, 1997). This might not be the case on every farm, as in order to use the system successfully, a similar amount of labor time might still be required. Based on our survey, the expectation of reduced labor is one of the main reasons why producers invested in automated feeders.

Dairy producers might be interested in purchasing automated calf feeders partly because of labor savings, but the ability to feed calves many times a day, a more natural behavior, is also an advantage. Our research team has collected data from many operations using automated feeders to document labor costs. It appears that labor time is not necessarily reduced, but the type of labor changes. Calves still need to be observed, pens cleaned, equipment cleaned and sanitized, etc. However, it would be very labor intensive to feed calves 4 to 6 times a day without automation.

An advantage of using the automated system compared to manually feeding calves twice a day is that the feeders allow for distribution of the total daily milk intake into small meals throughout the day, with no extra labor input, allowing a greater amount of milk to be fed without requiring the calf to drink a very large amount at each meal. These automated systems also can monitor the feeding behavior of each calf, such as number and timing of visits, the amount of milk consumed by each calf, and the number of unrewarded visits

(when no milk is fed), which has been shown in controlled research studies to help identify sick calves (Borderas et al., 2009).

Efficiency of automated feeders can be improved if the amount of time that each calf spends at the feeder in visits when it is not entitled to be fed is reduced. Feeding larger amounts of milk reduces the number of these unrewarded visits. In addition, automated feeding systems need to be managed properly to avoid competition. Potential strategies would be to keep group sizes relatively small, to properly introduce new calves to the group with adequate training, and to feed higher quantities of milk and in larger meals (4 times a day instead of 8 times a day). Many of these points were well addressed at this conference last year on a review by Steele et al. (2015).

Are any of the above mentioned strategies being successfully used on farms with automated calf feeders in the upper Midwest of the U.S.? There has been consistent growth in the upper Midwest U.S. on the number of farms installing computerized automated calf feeders. No research had been done in our region; therefore, we collected on farm data to learn what strategies are most common in typical Midwest herds. Automated calf feeders represent a new technology that needs study in order to understand housing and management characteristics that enhance calf welfare and dairy operation profitability.

This article summarizes some of the findings of a longitudinal field study we are conducting at the University of Minnesota involving 38 farms with calf feeders. These types of studies can provide descriptive information on housing and management practices, and by collecting many animal and facility measurements, we can identify factors that are associated with successful use of these systems. This methodology does not provide a direct 'cause and effect' connection, but we can identify guidelines and factors that can be important and then adopted by producers or investigated in more detail.

▪ Housing and Management Practices in the Midwest U.S. Automated Calf Feeder Facilities

Our study showed that 61% of the farms retrofitted an older facility (tiestall, pig barn, chicken barn, etc.) into a calf facility whereas the remaining 39% built a new barn specifically for the preweaned calves. We did not find a difference in calf health between new and retrofitted barns. Of these facilities, 50% were naturally ventilated barns, 39% were mechanically ventilated, 8% were additions to tunnel ventilated barns, and 3% were naturally ventilated "igloos." A great majority of facilities (87%) supplemented ventilation systems with positive pressure tubes. It is important that dairy producers work with an experienced engineer when designing a new barn or retrofitting an old one to

make sure all important aspects of ventilation and layout are properly considered.

The average number of calves per pen was 18.2 (Figure 1) which is less than the maximum suggested by the manufacturers (up to 30); the space per calf within the pen was 4.6 sq. meters. There was a wide distribution among farms.
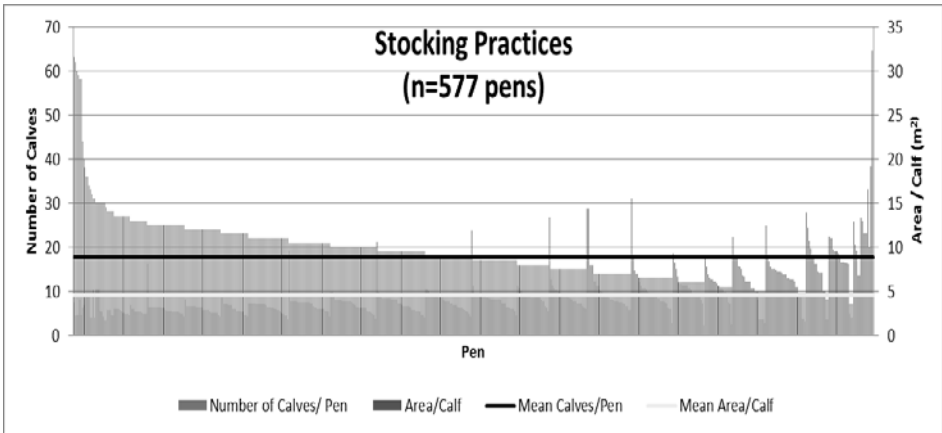

Figure 1. Stocking density as number of calves per pen and area per calf.

Average peak milk allowance was 8.3 liters per day and start milk allowance was 5.4 liters per day (Figure 2). A total of 68% of farms fed calves reconstituted milk replacer, 24% fed whole milk plus replacer or protein balancer, and 8% fed unsupplemented whole milk. Mean time from feeder introduction to peak milk allowance was 18 days.
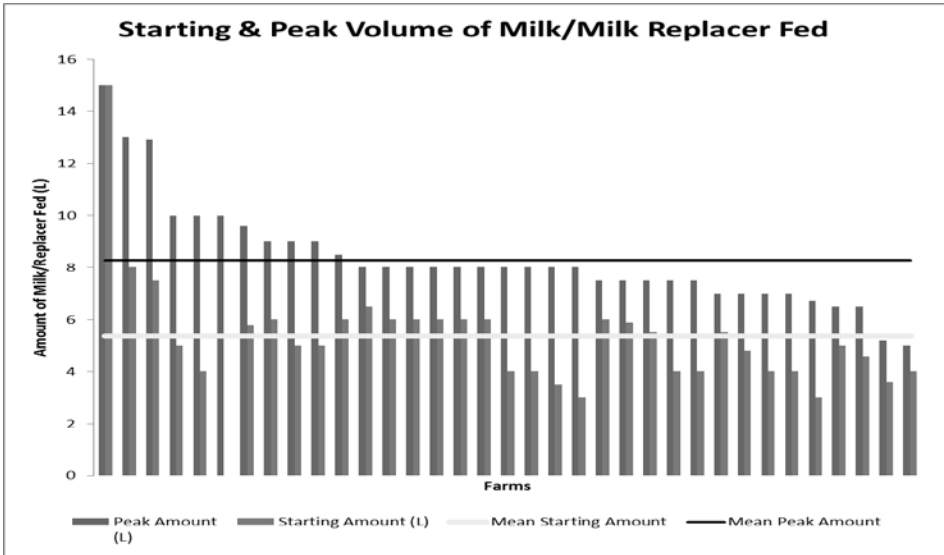
Figure 2. Starting and peak amounts of milk/milk replacer fed.

Calves were placed on the feeder group at 5.4 days of age (range of 0 to 14 days; Figure 3); 10 farms placed calves in the group at zero or one day of age. Placing calves on the feeder at a younger age requires more training and observation to make sure that calves are able to drink their required amounts of milk.
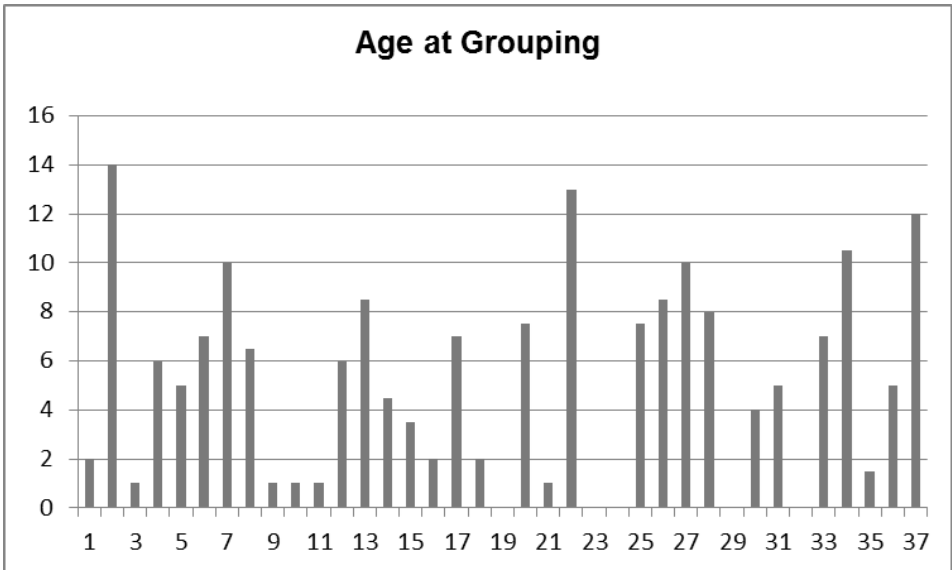

Figure 3. Age of introduction to group pen with automated calf feeder.

- ## **Calf Health Observations**

During each visit, calves (n=10,179) were scored for health by a single observer using four categories: attitude (0–4), ears (0–4), nose (0–3), eyes (0–3), and cleanliness (an indicator of diarrhea, 0–2), with 0 representing a normal, healthy calf. Body temperature was measured if a calf had an abnormal health score. In addition, blood was drawn from any calves one to five days old (n=985) and serum protein concentration used to assess passive immunity transfer. Milk samples were collected from the mixing container inside the feeder and at the end of the hose (tube) nearest to the nipple for measurement of standard plate count (SPC) and coliform count.

Figure 4 summarizes the calf health scores for the top 10th and the bottom 10th percentile farms. There was considerable variation among farms, indicating that housing and management factors can definitely influence the success of using these feeding systems. Table 1 summarizes the SPC and coliform counts for the top and bottom farms for the samples collected from the mixer and the hose (or tube). Again, there was a lot of variation and some very extreme numbers were detected. The milk the calf is drinking should have less than 100,000 CFU/ml for total plate count.
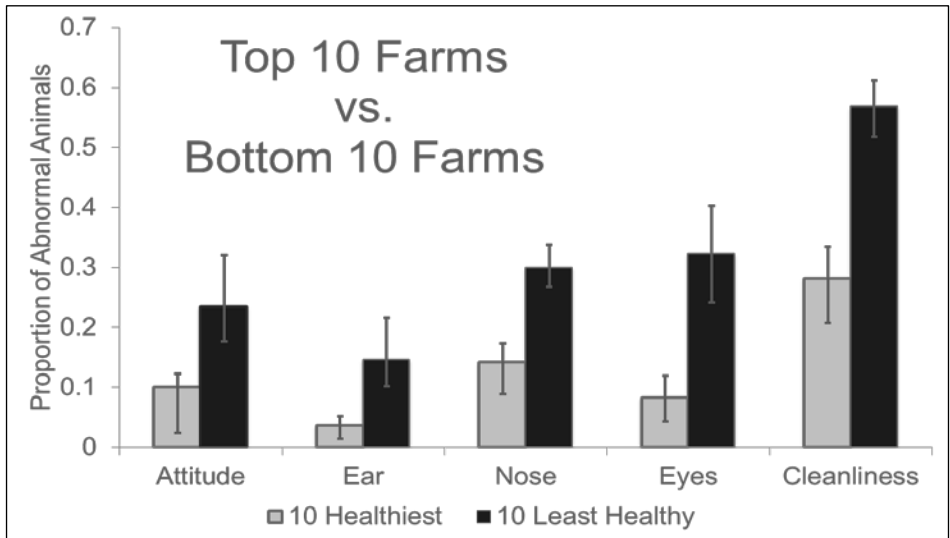

Figure 4. Average proportion of abnormal health scores.

Table 1. Farm average bacterial counts (cfu/ml) across visits for top and bottom 10 farms.

Item	Tube Coliform	Mixer Coliform	Tube SPC	Mixer SPC
Median of Top 10 (Q1-Q3)	887 (206-1,211)	12 (3-15)	87,590 (32,603-134,940)	9,006 (2,308-9,392)
Median of Bottom 10 (Q1-Q3)	5,659,567 (1,198,059-14,344,063)	522,263 (64,564-20,001,213)	21,140,625 (18,644,538-71,642,610)	10,209,920 (3,204,500-43,673,293)

▪ Risk Factors for Abnormal Health Scores

We conducted a mixed model statistical analysis to investigate the association of various housing and management factors with calf health. The factors listed below were associated with abnormal health scores; therefore, farms that have these characteristics are more likely to have more sick calves and be less successful using an automated calf feeder system.

▸ Number of calves per group: farms with greater numbers of calves per group had a higher number of sick calves.

▸ Space per calf: less space per calf was associated with higher number of abnormal scores. This was independent of group size. What this means is that a small group size with not much space available to move around the pen could still be a problem. This would be an important consideration when determining the pen size.

▸ Time to reach peak milk allowance: farms that waited longer to reach the maximum amount of milk had worse health scores. Most farms increased the amount of milk incrementally rather than offering a large amount of milk from day one. That is a good management practice, but the analysis indicated that it is better to achieve the peak amount in a shorter number of days, for example 8 days instead of 18 days. Plane of nutrition is important.

▸ Air speed in resting area and at the feeder: faster air movement at the resting area was associated with worse nasal scores, an indicator of respiratory disease; air speed at the feeder was associated with abnormal ear scores. This result can be an indication that ventilation is important, but drafts are undesirable.

▸ Standard bacterial plate count (SPC) on hose (tube) milk samples greater than 100,000 cells per ml: higher counts were associated with higher number of calves with abnormal health scores. We need to provide high quality, clean milk to calves.

▪ **Why Use an Automated Feeder?**

Dairy producers were asked the top reasons for purchasing the automated calf feeder. In order of priority, their top responses included:

1. less time spent on menial tasks

2. improved calf growth rate

3. improved information on calf feeding

4. natural diet changes/ more natural feeding

5. improved labor condition

6. reduced labor cost

7. social interaction between calves

8. ability for calves to express natural behaviors

▪ **Conclusions**

Automated calf feeders are growing in popularity and this trend will probably continue as producers want more flexible labor management and consumers want animals to have a more natural life. Feeding calves in groups allows calves to express some natural behaviors that cannot be expressed when housed individually, but offers some challenges in relation to maintaining good health, another important aspect of good animal welfare.

It was interesting to learn that producers might not be aware of the need for cleaning the equipment on a routine basis, which resulted in a wide distribution in the cleanliness of the milk that the calves were drinking across farms. It is extremely important to run all the circuit cleaning as recommended by the manufacturer (or more), replace hoses and nipples regularly, use a good disinfectant (such as chlorhexidine) to remove biofilms from the surfaces, keep the area around the feeder clean, provide clean and dry bedding to the calves, have good quality milk, calibrate the equipment to deliver appropriate concentration of nutrients and temperature for the milk, etc.

Good health is certainly achievable when using automated calf feeders to raise preweaned calves as long as appropriate management and maintenance are emphasized and implemented.

Acknowledgments

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Past, Present and Future of Footbaths in Alberta

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▪ Take Home Messages

- Footbaths are an essential component of claw health management.
- Footbath location should allow undisrupted cow flow, and preferably allow passage of young stock, dry cows and new additions to the herd.
- Footbaths should have proper dimensions.
- Not all products available are equally effective.
- Not all products available on the market have proven efficacy.
- Not all products work effectively when contaminated with manure.
- Adequate frequency of use and product concentration are essential.

▪ Introduction: The 5 W's of Footbaths in the Dairy Industry

Who

Worldwide, footbaths are considered to be an important component of a preventive claw health program. They play an important role in controlling potential contagious hoof disorders such as digital dermatitis (DD; aka hairy heel warts, strawberry heel warts, Mortellaro's disease). With the dairy industry moving away from tiestall housing, management in freestall housing with slatted or concrete flooring has lead to a more frequent occurrence of

infectious claw lesions. Although footbath products are not registered for treatment of claw diseases, they are sometimes used as a curative measure.

What

What is a footbath? Although the term "bath" makes us think the feet are bathed in a solution, dry footbaths have also been introduced. Mostly, footbaths are referred to as solution filled baths aiming for cleaning and disinfecting the feet of cows.

Where

Where can footbaths be used? Footbaths are an important component of the management of both pastured cows and cows housed indoors. The first step in installing a footbath is to determine the location of the bath; it must allow for undisturbed cow flow. Most often a position is chosen where cows exit the milking parlor; in pasture settings, outdoor locations can be identified too. These locations are chosen where all cows need to pass on a daily basis, without the opportunity to avoid the footbath. To allow for easy use of footbaths, filling and draining ease must also be considered when identifying the location. Preferably, cows should enter the footbath with minimal manure on their feet, and exit onto a clean floor to allow the product to work on clean skin. The use of footbaths for non-lactating animals or animals newly added to the herd might be a driver to identify an alternative location that allows for heifers and dry cows to walk through the bath.

The size of the footbath is also very important. Ideally the solution in the bath should reach the skin of the foot over the coronary band. This meets the recommendation of a footbath solution depth of approximately 10 cm (4 inches) when the last cow walks through the footbath.
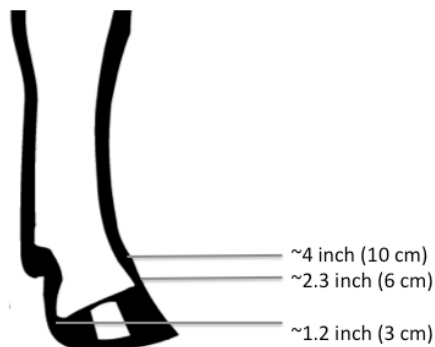

~4 inch (10 cm)
~2.3 inch (6 cm)
~1.2 inch (3 cm)

Figure 1: the preferred depth of the footbath solution

University of Wisconsin researchers recommend a footbath 3.0–3.7 m long and 0.5–0.6m wide, with a 28 cm step-in height (Length: 10–12 feet, width: 20–24 inches and ~11 inch deep; Cook et al., 2012). These dimensions optimize the number of foot immersions per cow pass, while limiting the footbath volume. The bath should not be perceived by the cow as an obstacle; it should allow cows to freely step in and out of the bath. The footbath should have a textured bottom or otherwise provide enough grip (i.e. footbaths with a sponge) for the cows to safely walk through without the risk of slips and falls.

When

Footbaths should be used for the control of infectious claw/skin diseases and potentially for hardening of the claw horn. When footbaths are used in temperatures below 10°C, the efficacy of the product (e.g. formalin) will drastically decrease. This can be mitigated using warm(er) water and storing solution at room temperature. If the solutions sticks to the cow's claw skin, the interdigital space is likely back to body temperature within 3 minutes.

If the solutions are left overnight or between milkings in colder temperatures, reduced efficacy should be anticipated. In high temperatures solutions might evaporate or precipitate, also negatively impacting the efficacy. A general recommendation is to refresh the solution after 200 cow passes. This number is somewhat variable and should be optimized for each farm, taking into account herd size, contamination with organic material, temperatures etc.

Why

Through the use of footbath solutions, cow's claws can be disinfected. This is needed in the control strategy for infectious claw diseases. Certain solutions like formaldehyde may help in hardening the horn of the claws, thus making the claws more resistant to non-infectious claw disorders (Arkins et al., 1986).

▪ Use of Footbaths in North America

Past

As was recently reviewed by Barkema et al. (2015), in North America, dairy cows are predominantly housed in tie- or freestall barns. Although there are considerable regional differences in housing type, a significant but decreasing proportion of dairy herds are housed in tiestalls. With increasing herd size and where freestall barns are the predominant housing system, an increase in infectious claw diseases is commonly observed, resulting in the need for preventive measures like footbaths.

Present

In 2011-2012, Dairy Farmers of Canada, along with Alberta Milk and ALMA, funded a research proposal investigating cow longevity and comfort. Part of this assessment focused on lameness and lameness management. In Quebec, Ontario and Alberta, a footbath was routinely used on 122 farms, which represented 87% of all farms visited. Smaller herds (below 100 cows) were less likely to use a footbath (Solano et al., 2015). One to 4 products were used per farm, the most common being copper sulfate and formaldehyde, with median concentrations of 4.5% (ranging from 0.3 to 12.5%) and 5% (ranging from 1 to 10%), respectively. On farms where footbaths are present, half of the farms visited used footbaths two days/week and the other half used them more than two days. Footbaths were more frequently used on farms with lower lameness prevalence (Table 1).

Table 1. Footbath management variables for dairy herds with low, medium and high lameness prevalence (Solano et al., 2015).

	Herd level lameness prevalence			
	Low (≤ 10%) n = 24	Medium (10 - 30%) n = 94	High (≥ 30%) n = 23	Overall n = 122
Footbath product (%)				
Copper sulfate	38	38	35	37
Formaldehyde	5	16	20	15
Copper & formaldehyde	52	40	35	41
Other	5	6	10	7
Number footbath products (%)				
1	29	41	35	38
2	57	44	50	47
≥3	14	15	15	15
Footbath frequency of use (%)				
≤ 2 d/wk	38	51	50	48
> 2 d/wk	62	49	50	52
Footbath dimension (median)				
Length (cm)	186	186	220	208
Width (cm)	76	72	73	74
Depth (cm)	15	16	15	15

Note: the overall category excludes 19 farms that had a footbath, but rarely used it

Cook et al. (2012) studied freestall-housed dairy herds with an average herd size of 1023 milking cows in 5 different countries (US, Spain, Japan, UK and New Zealand), and found that footbaths were used 1–4 times per day for 1–7 days per week, with between 80 and 3000 cows passing through the bath between changes of the chemical solution. Similar to that found in Canada, the most common agents used were copper sulfate (63%) and formalin (34%). Twenty-seven herds (42%) used more than one chemical. The median footbath was 2.03 m long by 0.81m wide (80 x 32 in), and was filled to a depth of 0.11 m (4.3 in).

▪ Efficacy of Footbaths

Footbaths Used in a Standardized Manner

We performed a follow-up study on 9 Alberta farms to evaluate what happens to the prevalence of DD if a standardized footbath is implemented. The prevalence of DD in the lactating herd was followed over a period of 4 months. DD scoring was done every 2 weeks in the milking parlor and confirmed with the observations in the trimming chute. For DD diagnostics we used the so-called M-score, that classifies the foot in 5 categories: M0 (no lesions), M1 (small early lesion), M2 (larger active lesion), M3 (healing lesion), M4 (chronic lesion) and M4.1 (chronic lesion, with an active M1 lesion) (Berry et al., 2012). Two months after the start of the study an automated footbath was implemented, with a computer-based weekly protocol of 2 consecutive days (4 milkings) using 5% copper sulfate. Controlled concentration and programmed refreshing of footbath solutions resulted in a decrease in DD on these dairy farms. All farms had a significant increase in M0 scores from 31 to 40%, whereas cows diagnosed with active M2 lesions transferred to more chronic stages of the infection (M3-M4). Cows affected with active M2 lesions decreased from 41 to 25%, whereas M3-M4 lesions increased from 28 to 35%. This shows that when footbaths are well designed and carefully used, the prevalence of active DD lesions will decrease dramatically (Student presentation Laura Solano; WCDS 2015).
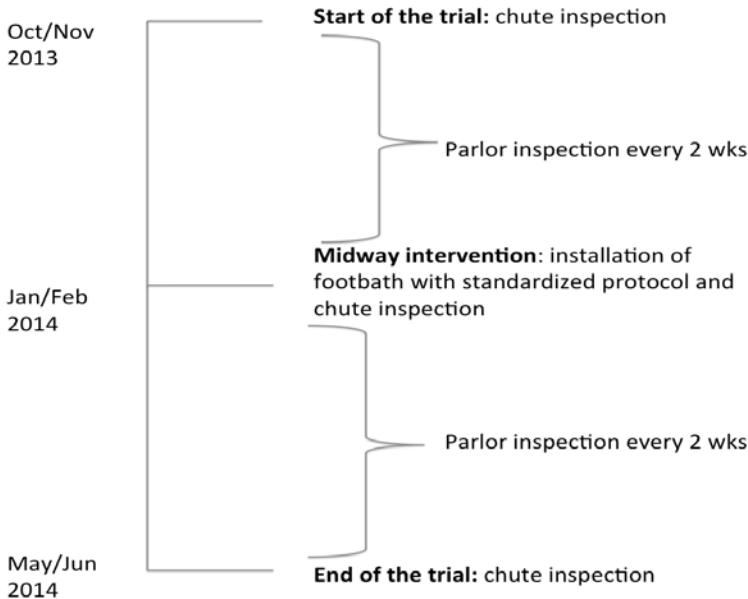

Figure 1. Timeline for DD prevalence study with implementation of standardized computerized footbath protocol.

Footbaths Used More Frequently

Our group performed another study on 10 Alberta dairy farms; 5 farms were assigned to an intensive copper sulfate protocol (5% solution, once a day, Monday–Friday), and 5 farms did not change their previous footbath protocol (non-interference). We scored the DD lesions of hind feet of all lactating dairy cows, every 3 weeks in the 5 times/week group and every 6 weeks in the non-interference group, respectively. Scoring was done in the milking parlor using the M-scoring system of Berry et al. (2012).

The farms that used the intensive copper sulfate footbath protocol experienced a decrease of all DD lesion stages and maintained a low prevalence of active lesions compared to farms with less specific and less frequent protocols irrespective of product used. Optimal frequency of footbath use to maintain low DD occurrence appeared to be >2/week, regardless of product used. This frequency results in active DD occurrence equal to the intensive copper sulfate protocol (Student presentation; Casey Jacobs WCDS 2014).
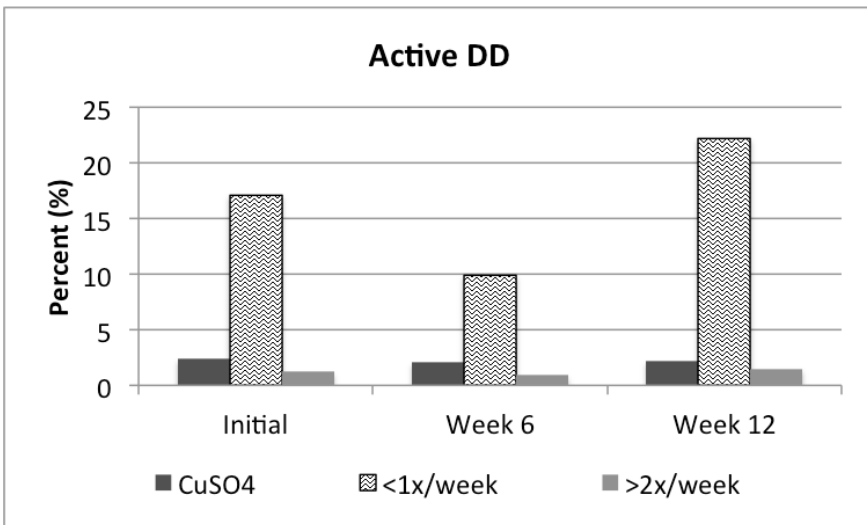

Figure 2. The impact of frequency of footbath use on prevalence of active DD lesions.

Our results are in line with a study conducted in the UK in 2012. Comparing the impact of weekly footbathing versus every other week with 5% copper sulfate, none of the cows had active DD lesions (M2) at the end of the study; however, in the group that was treated weekly, more cows had fully cured (M0) and had no lesions at all. When comparing a biweekly to a monthly application of copper sulfate, significantly more cows had active lesions in the

monthly treatment, indicating that more frequent use is necessary (Speijers et al., 2012). In an additional study from the same research team comparing both copper sulfate and hypochloride to no treatment, copper sulfate was the only footbath solution that was consistently effective for treatment of DD (Speijers et al, 2010).

▪ Future

Examples of Published Field Studies on Footbath Products

With concerns over use of antibiotics, environmental and carcinogenic impact, many new products are currently being tested in the field. Smith et al. (2014) determined the effect of a tea tree oil and organic acid footbath solution (Provita Hoofsure Endurance) on DD in dairy cows. The tea oil product resulted in similar outcomes compared to copper sulfate and the authors concluded that both products effectively reduced the active DD comparing the start to the end of the trial (Smith et al., 2014).

Teixeira et al. (2010) studied the effect of Dragonhyde, a commercially available disinfectant, and found that Dragonhyde performed better than formalin and that there was no difference between copper sulfate and Dragonhyde.

Besides environmental concerns, risks for farm workers have also been identified as a side-effect of footbath use. Doane at al. (2014) evaluated the exposure of farm workers to formaldehyde through the use of formalin footbaths. Although fumes were formed, the measured formaldehyde concentrations were falling within the safety guidelines established by the Occupation Safety and Health Administration (OSHA) of the United States and not perceived harmful for the farm workers.

Finally, with the increase in automated milking systems (robotic milking), alternatives to footbathing are explored, like foot sprays. The aim of foot sprays is comparable to footbath solutions; disinfection of the hoof and skin of the foot.

A Laboratory Approach Towards Testing Effectiveness of Footbath Products

Many new products are currently being tested in the field, exposing cows to new products without proven efficacy. The results, although published, are often hard to compare as experiments are evaluated differently and also products and concentrations used vary.

Therefore, funded by ALMA and Alberta Milk, our research team is currently working on a project together with the University of Wisconsin (Dr. D. Döpfer) examining techniques to test footbath products in the laboratory. In this study, concentrations of footbath products are determined that both inhibit as well as kill bacteria that cause DD. We are also testing the impact of manure in the footbath. This laboratory testing will help identify products with the highest potential. Ultimately, a field trial on dairy farms in Alberta should be the next step; i.e., testing these most promising chemicals under field conditions. With that knowledge, we can improve prevention and control strategies.

A study from the team in Wisconsin (Kulow et al., 2015) determined the ability, in the laboratory, of the product Thymox to kill or inhibit various species of microorganisms associated with infectious causes of bovine lameness. This product was identified as an environment and worker friendly product. The team found that this disinfectant has the potential as an alternative antibacterial agent for footbaths. However, field trials are needed to determine its effectiveness for the control and prevention of infectious claw diseases.

▪ Conclusion

On farm, the right location for a footbath needs to be determined allowing undisturbed cow flow. The footbath should have proper dimensions so all feet are immerged. If possible, the cow's feet should be clean before she steps into the footbath and out onto a clean and dry floor. Finally, the right concentration of the right product needs to be used in a high enough frequency to allow for proper disinfection to help prevent infectious claw diseases.

▪ **Good Resources**

Websites

http://dairy.ahdb.org.uk/resources-library/technical-information/health-welfare/footbathing-and-lameness-effective-management-for-dairy-cows/#.VoRZp5MrJZ0

http://dairyhoofhealth.info/

Books

Hoof signals: http://www.roodbont.nl/en/bookshop/15_Koesignalen/96-228_Hoof-Signals

Bovine Laminitis and Lameness: A hands-on approach
Edited by: Paul R Greenough, FRCVS
ISBN: 978-0-7020-2780-2

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Mechanisms Linking Postpartum Metabolism with Reproduction

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▪ Take Home Messages

- The reproductive and immune systems of the cow must function within the highly metabolic environment required to produce large volumes of milk in early lactation.

- The endocrine axes controlling reproduction respond to the hormones and metabolites that control the highly metabolic environment.

- Dairy cows are immunosuppressed after calving and this immunological state predisposes the cow to common diseases such as metritis and endometritis that can negatively affect reproduction.

- Genetic selection for high-producing healthy cows should reverse current trends and create a future cow that can transition well and reproduce successfully in a highly metabolic environment.

▪ Introduction

Genetic selection has successfully increased milk production per cow (Berry et al., 2015). The increase in milk production per cow has been achieved by selecting for large cows with the capacity to consume and metabolize large volumes of feed. The modern cow is highly metabolic. Her great capacity to metabolize nutrients is supported by endocrine systems that control the flow of metabolites to the mammary gland for the synthesis of milk (Lucy, 2008). Genetic selection for traits other than milk production was not done during the latter half of the 20th century (VanRaden, 2004). The resulting cows were compromised with respect to health, reproduction, and longevity, perhaps because the highly metabolic environment that supports high production was incompatible with other processes that were not under genetic selection (Berry et al., 2015). This review will discuss the relationship between

metabolism and reproductive function in early postpartum cows during the transition period.

▪ General Aspects of Metabolism Postpartum

An early lactation cow will produce 50 to 100 kg of milk per day. The cow undergoes a series of homeorhetic mechanisms to support the increase in milk production (Bauman and Currie, 1980; Bell, 1995). Several hormones are involved, but perhaps the best studied hormone is growth hormone (GH). Blood concentrations of GH increase shortly after calving (Lucy, 2008). The increase in GH orchestrates the homeorhetic mechanism that typifies early lactation. Growth hormone stimulates hepatic gluconeogenesis (glucose synthesis in the liver) to increase glucose supply to support that rapid increase in milk production shortly after calving. At the same time, GH antagonizes insulin action and creates an insulin resistant state so that circulating glucose cannot be used by liver, muscle or adipose tissue for the creation of glycogen or fat. Growth hormone also stimulates lipolysis. The mobilized lipid can either be incorporated directly into milk fat or used as an energy source in the postpartum cow. The end result is a large mass of glucose created through gluconeogenesis and fatty acids mobilized from lipid that are directly available for the synthesis of milk.

The Liver Coordinates the Homeorhetic Mechanisms Postpartum

A variety of tissues are involved in coordinating homeorhetic mechanisms that support milk production, including the brain (hypothalamus and pituitary), other endocrine glands (thyroid, adrenal, pancreas, etc.), the digestive tract (rumen, small and large intestines), adipose tissue (abdominal and subcutaneous stores), skeletal muscle, immune systems and liver. No endocrine gland or tissue can function alone to support the metabolic state of early lactation. This explains why the entire animal must be healthy to achieve high milk production. Although we traditionally thought that hormones controlling lactation arose exclusively from traditional endocrine glands, we now know that most tissues produce hormones with the capacity to control various aspects of the physiological state. This includes the liver, which functions as a highly metabolic organ with important endocrine functions.

Among the tissues that support milk production, the liver is pivotal because it coordinates nutrient metabolism with the endocrinology of the cow (Figure 1). There is a decrease in GH receptor expression in liver before calving. The decrease in GH receptor expression before calving is associated with a decrease in the release of insulin-like growth factor–1 (IGF1) from the liver. IGF1 is the primary negative feedback molecule for GH. The decrease in IGF1 from liver, therefore, explains the increase in GH that occurs early postpartum. The increase in GH early postpartum causes the increase in

gluconeogenesis and insulin resistance that supports the demand for large amounts of glucose postpartum. The increase in GH also drives lipolysis that mobilizes fatty acids (NEFA) from adipose tissue for incorporation directly into milk fat or for the generation of cellular energy. Incomplete metabolism of NEFA leads to an increase in blood ketone concentrations (primarily beta hydroxybutyrate or BHB).
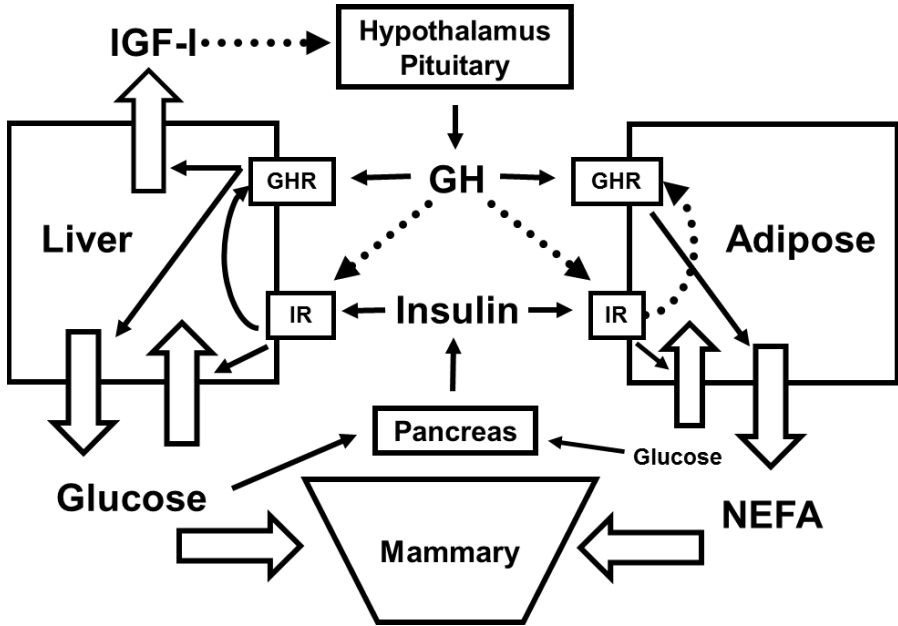

Figure 1. Model for the interaction of growth hormone (GH) and insulin in postpartum dairy cows (Lucy, 2008). Solid lines infer stimulatory actions. Broken lines infer negative feedback or inhibitory actions. Growth hormone signals in liver and adipose through its receptor (GHR) and inhibits the activity of the insulin receptor (IR). Early postpartum and high producing dairy cows have high GH, low insulin, and peripheral insulin resistance. The condition promotes glucose and NEFA availability for milk synthesis. Later lactation and low producing cows have lower GH, higher insulin, and greater insulin sensitivity. The later condition reduces NEFA mobilization and shunts glucose to peripheral tissues (including adipose). See text for specific details and the endocrine sequence of events in postpartum cows.

Despite these physiological mechanisms, the cow becomes hypoglycaemic after calving because glucose demand drives blood glucose concentrations downward (Lucy et al., 2014). The hypoglycaemia keeps blood insulin concentrations low. Low blood insulin maintains the state of low liver GH receptor expression and low circulating IGF1. The low circulating IGF1 keeps GH secretion high because there is no negative feedback on GH. The low concentrations of glucose have an additional consequence; specifically, inadequate glucose supply contributes to the incomplete oxidation of NEFA, which creates elevated BHB postpartum (White, 2015).

The Shift from a Catabolic to an Anabolic State Postpartum

The preceding section describes the catabolic state of early lactation. The endocrine state of early lactation (high GH, low IGF1, low insulin, low glucose, and high NEFA and BHB) remains in place until the cow progresses toward a positive energy balance (anabolic state). A key regulatory molecule is glucose (Lucy et al., 2014). In time, the cow's ability to generate glucose through the expansion of digestive capacity postpartum, a greater capacity to consume and digest nutrients, and greater gluconeogenesis leads to an increase in glucose supply. The cow also passes peak lactation so that the demand for glucose is less. The increase in glucose supply relative to demand increases available glucose and stimulates insulin secretion. Greater insulin secretion causes an increase in GH receptor expression (Butler et al., 2003). The increase in GH receptor expression causes an increase in IGF1. The increase in IGF1 feeds back negatively on GH. The reduction in GH postpartum relieves the insulin resistance so that excess glucose can now flow into other tissues. This shift in glucose flow is equivalent to the shift from a catabolic state to an anabolic state postpartum. A cow that is catabolic can begin to restore glycogen in liver and muscle and also gain adipose tissue mass.

The shift from a catabolic to an anabolic state is important relative to the body condition score (BCS) of the postpartum cow. The exact mechanisms are unclear but cows that maintain greater BCS postpartum are generally better with respect to reproduction (Kawashima et al., 2012).

▪ Linking Metabolism to Reproduction Postpartum

Scientific thinking about the link between metabolism and postpartum reproduction has progressed far beyond the traditional notions of negative energy balance and interval to first ovulation. It is clear that there is a complex interplay between the endocrine systems controlling metabolism, the endocrine systems controlling the ovary, the endocrine system within the ovary itself, and the immune system of the cow.

Postpartum Reproduction Starts with a Healthy Liver

Perhaps the most-important first step toward maintaining good reproduction on a dairy is to maintain a healthy liver in transition dairy cows. Maintaining a healthy liver is best achieved through appropriate dry cow and transition cow management to maintain an appropriate BCS at calving and prevent excessive BCS loss after calving. Appropriate dry cow nutritional management is essential (Drackley and Cardoso, 2014). An appropriate BCS at calving cannot be underemphasized. Cows with excessive BCS at calving and excessive BCS loss after calving develop fatty liver postpartum. The sequence of deleterious events associated with fatty liver are depicted in Figure 2. Cows that have excessive BCS at calving typically develop fatty liver because of poor intake postpartum. The poor intake postpartum can be explained by insufficient appetite perhaps caused by the excessive BCS. Failure to consume adequate feed leads to excessive adipose tissue loss and elevated NEFA in blood. The NEFA enter the liver but cannot be fully metabolized so fat builds up in liver tissue. Fat causes inflammation in liver. The inflammation associated with fatty liver inhibits liver metabolism and gluconeogenesis (Garcia et al., 2015). Cows with fatty liver are incapable of achieving the high rates of gluconeogenesis that are needed to maintain adequate glucose supply (McCarthy et al., 2015). The problems that begin with inadequate intake, inflammation and poor liver health, therefore, eventually affect the entire metabolic make-up of the cow.

Linking Liver Health to Reproduction

There are a number of consequences to fatty liver that go beyond the immediate damage of the liver tissue. The abnormal metabolic and hormonal environment created by the inflamed and damaged liver can affect not only the capacity for the cow to consume feed and make milk but also the capacity of the cow's immune system to combat disease (Zerbe et al., 2000) as well as the capacity of her reproductive axis to function normally (Clarke, 2014).

Postpartum Immunology

The current theory is that the metabolic environment in postpartum cows suppresses the innate immune system through effects on the function of polymorphonuclear neutrophils (PMN; Graugnard et al., 2012; LeBlanc, 2012). Changes in circulating concentrations of nutrients and metabolites that occur normally in the postpartum cow are exactly opposite to those that would benefit the function of PMN. In extreme cases, like those seen for fatty liver or ketotic cows, the shifts in hormones and metabolites are greater and there is the potential to compromise immune function further. For example, glucose is the primary metabolic fuel for PMN (Moyes, 2015). There is good agreement between in vitro analyses of PMN function and epidemiological evidence that indicates that an abnormal metabolic profile during the periparturient period
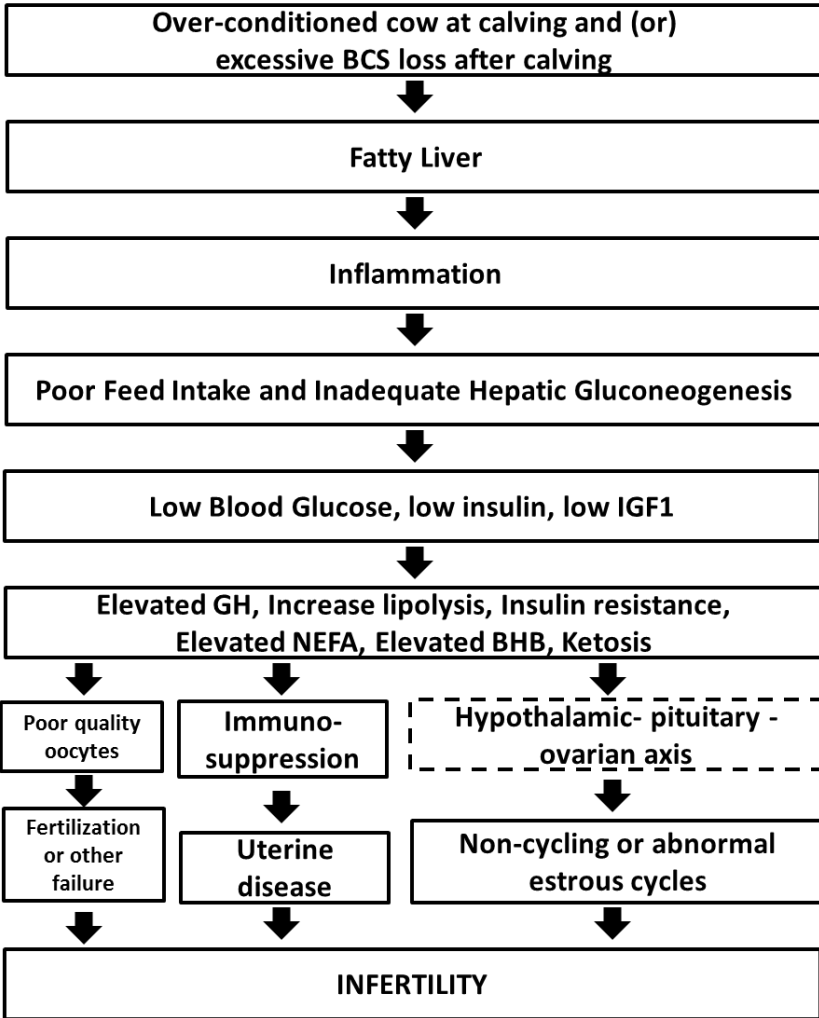
Figure 2. Sequence of events that link excessive body condition score (BCS) at calving and (or) excessive BCS loss after calving to infertility later postpartum. The dashed line around the box for "hypothalamus-pituitary-ovarian axis" indicates in inhibitory effect of the metabolic profile. See text for specific details and the sequence of events in postpartum cows.

predisposes the cow to uterine disease during the early postpartum period and infertility later postpartum (Chapinal et al., 2012; Esposito et al., 2014; Wathes, 2012). A plausible hypothesis is that the abnormal metabolic profile of the postpartum cow creates immunosuppression. This immunosuppression

leads to a poor response to uterine infection. The poor response to uterine infection can lead to metritis in the short term and subclinical endometritis in the long term. Subclinical endometritis leaves a permanent "scar" on uterine tissue that remains after the disease state is seemingly resolved (LeBlanc, 2012). The nature of scar left by subclinical endometritis is unknown but clearly creates the risk for infertility and early embryonic loss later postpartum.

The cells that respond to uterine infection (predominantly PMN) are the same cells that combat the organisms that cause mastitis and pneumonia (2 additional common diseases of the postpartum cow). These diseases do not directly affect reproductive tissues but secondary responses of the cow to the disease can disrupt the estrous cycle and cause embryonic loss. In a recent study, Fuenzalida et al. (2015) found that a mastitis event during the breeding period was associated with lower fertility. Cytokines and other hormones released by the inflamed mammary tissue can circulate throughout the cow and block ovulation or cause premature regression of the corpus luteum (Sheldon, 2015).

Cows that fail in the fresh cow pen may do so because their compromised immune system fails to overcome the initial challenge from pathogens. There is perhaps a "tipping point" beyond which a cow cannot recover from infection. With respect to immune system function early postpartum, an appropriate metabolic response to early lactation may maintain adequate immune cell functionality so that the tipping point is not reached.

Restoration of Ovarian Activity

The traditional focus for reproductive biologists studying dairy cows postpartum has been interval to first ovulation. This is because in traditional dairy systems the non-cycling cow was a major concern. The interval to first ovulation depends on the initiation of luteinizing hormone (LH) secretion from the pituitary. The secretion of LH depends on the release of gonadotropin-releasing hormone (GnRH) form the hypothalamus. LeRoy et al. (2008) concluded that glucose and insulin were the most likely molecules to exert an effect on GnRH secretion in the postpartum dairy cow. The most important actions of insulin and IGF1 are observed when either hormone acts synergistically with the gonadotropins [either follicle stimulating hormone (FSH) or LH]. This strong synergism explains the well-established relationship between circulating concentrations of insulin and IGF1 and the interval to first postpartum ovulation (Kawashima et al., 2012; Lucy, 2011; Velazquez et al., 2008). In general, improved metabolic indicators are associated with an earlier interval to first ovulation.

Restoration of "normal" Ovarian Cycles

Recent studies have demonstrated that cows may not cycle "normally" after first ovulation (Remnant et al., 2015). Abnormal estrous cycles include short

cycles, long cycles with normal luteal phase progesterone concentrations, long cycles with subnormal luteal phase progesterone concentrations, and failure to ovulate with one week after luteolysis. The same hormones that control when the cow begins to cycle (insulin, IGF1, FSH, and LH) also have an effect on cyclicity, which relates to the functionality of the follicle and corpus luteum. The hormonal environment created by lactation (in this example low blood glucose, insulin and IGF1 concentrations) may potentially affect the capacity for ovarian cells to respond to gonadotropins (FSH and LH). In the cycling cow, this could potentially affect estradiol production by the follicle as well as progesterone production by the corpus luteum. Common problems that are encountered in lactating cows, for example, poor estrus expression (presumably caused by inadequate estradiol production by the follicle; Woelders et al., 2014) and sub-optimal luteal phase progesterone (inadequate progesterone production by the corpus luteum; Wiltbank et al., 2011) could be explained by the fact that the cow has inadequate insulin and IGF1 to synergize with FSH and LH to maintain steroidogenesis by the ovary. The manifestation of this biology at the level of the cow may be a series of abnormal estrous cycles that largely go undetected by the producer because they are difficult to track in cows with poor expression of estrus. Part of the success of ovulation synchronization programs that are used widely in some countries can be explained by effectively overcoming abnormal patterns of estrous cyclicity that typify postpartum dairy cows (Wiltbank and Pursley, 2014).

Oocyte Health

The ovary has 2 functions in the postpartum cow. The first is an endocrine function (as described above) to produce a variety of hormones that include progesterone and estradiol. The second is to produce the female gamete (oocyte). The oocyte rests in a quiescent state within the ovary until approximately 2 months before ovulation. At that time, it initiates growth along with the surrounding granulosa cells. There is good evidence from several sources that the metabolic environment within which the oocyte develops can affect its capacity for fertilization and further development (Berlinguer et al., 2012; LeRoy et al., 2008; LeRoy et al., 2011). One theory is that the long development program of the oocyte before ovulation enables an irreversible imprinting of the metabolome on the oocyte itself. If this imprint is negative then this may explain why cows with metabolic disease early postpartum have infertility several months later.

▪ Solutions

As stated above, avoiding metabolic and other disease in transition cows should theoretically improve reproduction later postpartum. There is very strong evidence to support this point (Drackley and Cardoso, 2014). Avoiding problems in transition cows begins with appropriate management and feeding

of dry cows and continues through the management in calving pens, fresh cow pens and early lactation pens.

Ultimately, the genetics of modern dairy cows needs to be improved so that the cow possesses the underlying biological to sustain health and productivity in an extremely metabolic condition. The greater emphasis that is now placed on health, reproduction and longevity in most genetic indices should enable this genetic change to occur (Berry et al., 2015). The implementation of genomic technologies will shorten the time required to achieve the desired genetic change.

▪ Conclusions

The reproductive and immune systems of the cow must function within the highly metabolic environment required to produce large volumes of milk in early lactation. Unfortunately, years of genetic selection for milk production without consideration of other traits led to problems in health, reproduction, and longevity in modern dairy cows. One of the underlying reasons for the genetic problem was that the hormones and metabolites that control the highly metabolic environment were at odds with the endocrine axes controlling reproduction. The highly metabolic environment also leads to immunosuppression after calving and this immunological state predisposes the cow to common diseases such as metritis and endometritis that can negatively affect reproduction. Genetic selection for high-producing healthy cows should reverse current trends and create a future cow that can transition well and reproduce successfully in a highly metabolic environment. The implementation of genomic technologies will shorten the time required to achieve the desired genetic change.

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Diagnosing Trace Mineral Deficiencies and Excesses in Transition Dairy Cows

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▪ Take Home Messages

‣ Trace minerals are required for normal functioning of all biochemical processes in the body. If minerals are adequate in the diet, but animals are found to be deficient, antagonistic interactive effects of other minerals need to be investigated.

‣ Deficiencies of essential trace minerals, depending on severity, can result in clinical or subclinical deficiency signs. These clinical signs may be very subtle and difficult to identify.

‣ Historically, testing for deficiencies has been performed on diets and/or dietary components to ensure "adequate" concentrations. However, general mineral analysis does not identify the chemical form of these minerals, which can dramatically alter their bioavailability and utilization. There are also many trace mineral antagonists with element-to-element interactions.

‣ Appropriate diagnosis of mineral status involves thorough evaluation of groups of animals. The evaluation should include a detailed health history, feeding history, supplementation history, and analysis of the appropriate sample from several animals for their mineral status.

▪ Introduction

Trace minerals are required for essentially all biochemical processes in the body. Many of these minerals are necessary for optimal growth, physiologic function, and productivity in animals. This paper focuses on 8 trace minerals: cobalt (Co), copper (Cu), iodine (I), iron (Fe), manganese (Mn), molybdenum (Mo), selenium (Se), and zinc (Zn). These trace minerals have been chosen because nutritional deficiencies or disturbances in their metabolism are relatively common, and substantial information is available about their

metabolism and the amount needed for optimal health and productivity in animals. Testing of blood, serum, or tissues for total mineral concentration is a popular and potentially valuable means of assessing trace mineral nutritional status, and is generally more practical than expensive functional approaches of measuring specific mineral-containing proteins or enzymes. Modern analytical techniques make blood and tissue trace mineral analysis practical and relatively inexpensive. Of particular importance is the recent application of inductively coupled plasma/mass spectroscopy (ICP/MS) analysis to the diagnostic evaluation of animal samples. This technique is fast, extremely sensitive, precise and accurate, and allows for the simultaneous measurement of a wide array of trace minerals (Herdt and Hoff, 2011).

Trace minerals play a key role in supporting immune function; therefore, maintaining adequate trace mineral status during the dry period is an important component in achieving good cow health around parturition, when the cow experiences significant metabolic and physiological changes and her immune system is stressed.

Direct measurement of trace mineral content in blood and tissue is subject to considerable limitations in evaluating nutritional status. Consider the assessment of trace mineral nutrient evaluation in animals as described by Suttle (2010) in Figure 1. This conceptual approach recognizes that during periods of inadequate dietary intake, depletion of storage pools and transport forms are well defined and accessible for measurement. This concept can be readily applied in clinical use. However, from a diagnostic standpoint, not all trace elements fit well into this scheme because for some there is no recognizable storage pool and for others the transport and functional pools overlap.
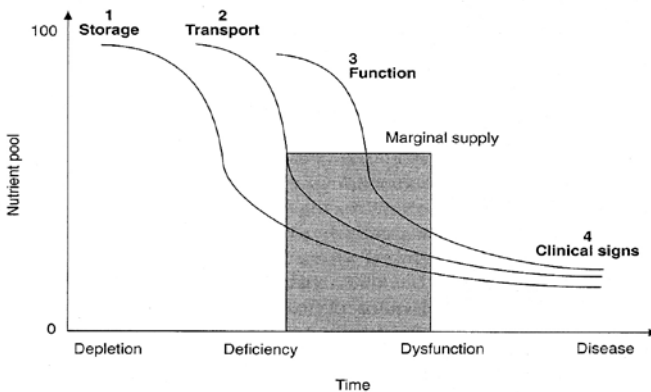

Figure 1: The sequence of pathophysiological changes that can occur in mineral-deprived livestock (Suttle, 2010)

Furthermore, factors other than nutrition are known to affect serum trace mineral concentrations. Most notably, homeostatic forces modulate the serum concentrations of most trace minerals within a range of homeostatic set points that vary in width among the different minerals. Other factors such as physiologic state (e.g., pregnancy, lactation, and gestation) may influence serum trace mineral concentrations. The presence of inflammation also has a large influence on serum concentrations of some minerals.

▪ Deficiency and Toxicity Diagnoses

Historically, testing for deficiencies has been performed on diets and/or dietary components to ensure "adequate" concentrations in the diet. However, general mineral analysis does not identify the chemical form of these trace minerals, which can dramatically alter their bioavailability and utilization. This is especially important when considering the increasing use of "chelated" minerals, as they can have significantly greater overall bioavailability than the inorganic minerals. Mineral deficiencies can be presumptively diagnosed by development of clinical disease or by post-mortem identification of tissue lesions. Proof of deficiency requires analytical verification because most deficiencies do not have unique clinical signs or lesions. Circumstantial proof of a deficiency may be provided by positive response to supplementation of a suspected deficient mineral. The problem is that a positive response may have nothing to do with the supplementation and may be just a time-responsive correction of some other clinical condition (Hall, 2015).

The action of trace minerals is dose dependent, and even essential trace minerals can produce toxic effects when consumed at high concentrations. The toxic effects of trace minerals can be subtle, with no clinical signs. For example, Lyman (2013) reported copper toxicity in all age groups of Wisconsin Holsteins, causing subclinical liver damage. A review of 225 WVDL submissions showed a mean copper level of 143 ppm (25-100 ppm is the recommended copper level for adult dairy cows).

Most of the trace minerals have several means of measurement for identification of deficiencies, but most have one that is more specific than the others. A good example is serum copper concentrations. Unless serum copper is at a critically low value, it has no significant predictive value in assessing potential for copper deficiency disease. Another example is the debate between serum and whole blood selenium values. Serum selenium represents the transport pool and is very sensitive to dietary changes and liver mobilization. On the other hand, whole blood selenium values represent both transport and a portion of the biochemical function pools. This measure is somewhat less sensitive to changes as a result of a greater proportion of whole blood selenium being present as the erythrocyte enzyme, glutathione peroxidase. If we were to assess a potential response to dietary change,

serum selenium values would respond within a day or so while whole blood, like liver values, may take a month or more to show a significant change. This could dramatically impact interpretation of the dietary response.

Liver mineral concentrations are good markers for the storage pool; however, they are not always highly associated with the presence of disease. Liver mineral concentrations may give us some insight into the adequacy of the mineral supplementation program and potential for disease. The assessment of mineral status in fetal and neonatal animals is quite different than adult animals. The fetus can concentrate trace minerals in its liver from the dam, and therefore the comparison to adult values is inappropriate. This is especially relevant for copper, iron, selenium, and zinc. We are currently developing databases determining normal trace mineral concentrations in the fetal and neonatal liver. Also, a few more predictive markers for specific nutrient pools need to be identified.

When individual animals are tested, their prior health status must be considered in interpreting the mineral concentrations in tissues. Infectious disease, fever, stress, endocrine dysfunction, and trauma can alter both tissue and circulating serum/blood concentrations of many minerals. Therefore, evaluation of multiple animals is much more reflective of mineral status within a group than testing individual animals that are ill or have died from other disease states.

▪ Live Animal Sampling

A variety of samples from live animals can be analyzed for trace minerals. Testing of blood, serum, or liver samples for total mineral concentrations is a popular and potentially valuable means of assessing mineral nutritional status that is generally more practical than the more functional approaches mentioned earlier. Other samples from live animals occasionally used for analysis include urine and milk. Hydration status significantly affects urinary mineral concentrations and the mineral content in milk varies through lactation and can be greatly affected by disease. Hydration status is not typically considered when evaluating whole animal mineral status.

Serum samples should be separated from the red/white blood cell clot within 1 to 2 hours of collection. If the serum sits on the clot for a longer period of time, minerals that are present in high levels in cells within the clot can leach into the serum and falsely increase the serum content. Minerals for which this occurs include iron, zinc, and potassium. In addition, hemolysis from natural disease or due to collection technique can result in falsely increased levels of manganese, selenium, and zinc.

The best type of tube for serum or whole blood mineral analysis is the royal-blue top vacutainer tube, as it is certified trace-metal free. The red-top vacutainer tubes can give abnormally increased results for zinc content as a zinc-containing lubricant is commonly used on the rubber stoppers.

Samples should be appropriately stored for adequate sample preservation. Liver biopsies, urine, and serum can be stored frozen long-term or refrigerated if mineral analysis is to be completed within a few days. Whole blood and milk should be refrigerated but not frozen, as cell lysis will compromise the integrity of the sample.

Liver biopsies, because of their small size, are susceptible to desiccation unless properly stored. These small biopsy samples should be placed into small tubes, with the sample pushed all the way to the bottom. Small 1-2 ml micro-centrifuge tubes work well for this (See AHL website, LabNote 19, http://www.guelphlabservices.com/files/AHL/AHL%20LabNotes/LabNote1 9.pdf). Placing the sample at the bottom of the tube minimizes the air-to-sample interface area and the potential for desiccation. The sample can then be frozen.

▪ Post-mortem Animal Sampling

A variety of post-mortem samples can be analyzed for trace minerals. Liver tissue is the most common tissue analyzed for mineral content, as it is the primary storage organ for many of the essential minerals. Post-mortem samples can be stored frozen until they are analyzed. Other samples, such as kidney, source material, feed, and water, may also be needed depending on the deficiency or excess suspected.

▪ Trace Mineral Functions and Bioavailability

Cobalt (Co)

Cobalt deficiencies have not been reported in Alberta, although cobalt levels in feed and livestock have not been widely studied in the province (www.agric.ab.ca). The only known function of cobalt is its role as a component of vitamin B-12. Ruminal microorganisms are able to synthesize vitamin B-12 from dietary cobalt. A lack of dietary cobalt for vitamin B-12 synthesis by rumen microorganisms can also alter ruminal fermentation. Deficiency is associated with decreased feed intake, lowered feed conversion, reduced growth, weight loss, hepatic lipidosis, anemia, immunosuppression, and impaired reproductive function (Herdt and Hoff, 2011).

Copper (Cu)

More than 90% of feed produced in Alberta is low in copper. Deficiencies occur through prolonged consumption of forages low in copper and/or the consumption of forages containing elevated levels of molybdenum or sulfur, which are natural antagonists of copper.

Copper deficiency is one of the most commonly encountered nutritional problems in ruminants, but copper excess is also commonly encountered in dairy cattle. Excessive copper is a relatively common finding in multiparous dairy cows, while most deficiencies are identified in calves and first lactation cows (Lyman, 2013; Hall, 2010). Copper is an essential trace element for livestock and has two functions. Copper is a component of a number of enzymes in which it serves a catalytic function. These enzymes are important for the structural integrity of collagen and elastin, detoxification of superoxide radicals, pigmentation, iron transport, and energy metabolism. Copper can also be a structural component in macromolecules, acting as a coordinating center.

Clinical signs of deficiency can appear as reduced growth rate, decreased feed conversion, poor immune function (failure to respond to vaccinations), impaired reproductive function, anemia, and rough, dull hair coat. Cows can deplete their own body reserves to ensure neonatal adequacy. Therefore, copper deficiency in calves would indicate that the dam is deficient and that she would likely also have poor colostrum quality, leading to inadequate neonatal protection even with adequate volume of colostrum.

The best method for copper evaluation is via analysis of liver tissue (storage pool) because depletion of hepatic copper is the earliest sign of inadequate copper consumption. Copper evaluation can be reliably determined on liver biopsy samples as small as 50 to 75 mg of fresh tissue. Such samples are easily obtained with Tru-Cut-style biopsy instruments (AHL website).

Deficiency in a herd will result in some animals that have low serum values, but serum content does not fall until liver copper is significantly depleted. In herds that have been sampled with liver biopsies and found to have a high prevalence of deficiency, it is not unusual to see a high percentage of "normal" serum copper levels (Hall, 2010). In Guelph, we have identified herds as "marginally deficient" from liver biopsies, and most of the cows have "normal" serum copper levels. Thus serum copper analysis should be viewed as a screening method only.

The recommended adequate wet weight liver copper concentration in adult cattle is 25 to 100 ppm. In comparison, a late-term fetal or early neonatal liver should have 65 to 150 ppm copper to be considered adequate.

Iodine (I)

The primary role of iodine is in the synthesis of hormones by the thyroid gland. Thyroid gland hormones regulate energy metabolism, reproduction, thermoregulation, growth and development, circulation, and muscle function. The levels of iodine in forages in Alberta are low and supplementation is necessary. Clinical signs, such as goiter, decreased milk yield, impaired fertility, and increased incidence of retained placenta, have been reported.

Evaluation of iodine is of interest because of the large potential for dietary deficiency, the possibility of toxicity, and the transfer of iodine to human food products, especially dairy products. Overt iodine deficiency is manifested as goiter, which is enlargement of the thyroid gland. Goiter may occur in utero and not be observed until birth. Congenital goiter may occur in the offspring of dams that are not themselves suffering from overt iodine deficiency. For post-mortem diagnosis of iodine deficiency, the tissue of choice is thyroid gland. Low iodine levels indicate iodine deficiency (Herdt and Hoff, 2011). At high iodine intakes, liver concentrations may increase more than normal, but hepatic concentrations are not useful in diagnosing iodine deficiency.

For the antemortem diagnosis of iodine deficiency, evaluation of thyroid function is the most suitable means of evaluation. This evaluation involves, at a minimum, the determination of serum thyroxine concentrations (T4) and ideally should include measurement of thyrotropin-releasing hormone and thyroid-stimulating hormone. Direct measurement of serum iodine is less sensitive and specific for the diagnosis of iodine deficiency.

Iron (Fe)

Iron is an essential nutrient that is required in a variety of metabolic processes and is found in all body cells. The largest portion is found as a necessary component of the protein molecules hemoglobin and myoglobin. Iron plays a vital role in the transport of oxygen by hemoglobin and in oxygen storage and transport in muscle by myoglobin. Iron is essential for normal cellular function of all cell types and is found in plasma (transferrin), milk (lactoferrin), and liver (ferritin and hemosiderin).

Deficiency of iron is of limited practical significance in farm livestock. Confinement increases the possibility of iron deficiency in young suckling animals, or animals reared on a diet of milk. Severe blood loss from parasites or other causes also produces secondary iron deficiency. A variety of factors in feeds can have enhancing or inhibiting effects on iron bioavailability. Trace mineral interaction may also alter bioavailability; for example, excessive dietary cobalt or manganese may interfere with iron availability.

Both liver and serum concentrations are commonly used to diagnose iron deficiency and toxicosis. When using serum to measure iron deficiency, samples that are hemolyzed should not be used. Interpretation of the iron status should be made with consideration of the overall health of the animal, as inflammation and infection can alter serum and liver iron concentrations.

Manganese (Mn)

Manganese is involved in a broad array of enzyme systems in the body and affects a wide variety of biochemical processes including carbohydrate, fat and protein use. Manganese is also involved in proper bone development and maintenance. Pasture grasses and legumes are typically good sources of manganese, whereas corn silage and cereal grains are poor sources (Herdt and Hoff, 2011).

Manganese deficiency in ruminants is associated with impaired reproductive function, skeletal abnormalities, and less than optimal productivity. Cystic ovaries, silent heat, reduced conception rates, and abortions are reported reproductive effects. Neonates that are manganese deficient can be weak, small, and develop enlarged joints or limb deformities.

Manganese at sub-normal to deficient concentrations is identified routinely in dairy cattle. The lower levels in dairy cattle may in part be the result of high levels of calcium and phosphorous in dairy rations, which can be antagonistic to the bioavailability of manganese. This is not seen in beef cattle (Hall, 2010).

Of the samples available, liver is the most indicative of whole body status, followed by whole blood, and then serum. Hemolysis can result in a false increase in serum content. Response to supplementation has frequently been used as a means of verifying manganese deficiency, but it is critical that a bioavailable form be utilized. For example, manganese oxide has very poor bioavailability.

Unlike for copper, selenium, iron, and zinc, late-term fetuses and neonates have lower manganese content than adult animals. Calves will generally have similar normal ranges to adults by 5 to 6 months of age. For wet weight liver manganese, normal adult range is 2.0 to 6.0 ppm whereas the neonatal normal range is 0.9 to 4.5 ppm.

Molybdenum (Mo)

Early nutritional interest in molybdenum was centered on its impact on copper availability in ruminants. An essential role for molybdenum came from the discovery that the flavoprotein, xanthine oxidase, contains molybdenum, and

that its activity depends on the metal (Suttle 2010). Although molybdenum is an essential trace mineral, the requirements are very low and clear signs of deficiency have not been seen in cattle. The tolerance of livestock to high molybdenum intakes varies with the species, the amount and the chemical form of the ingested molybdenum, the copper status of the animal, and the diet and the forms and concentration of sulfur and iron in the diet. Cattle are the least tolerant species. Growth retardation, weight loss, and anorexia are common, and diarrhea is typical only in cattle. Cattle have clinical signs that mimic copper deficiency if they have less severe exposure, as a result of formation of thiomolybdates in the rumen, which can diminish copper absorption and bind systemic copper and render it non-functional.

The assessment of molybdenum status is usually undertaken when there are concerns about molybdenum toxicity or conditioned copper deficiency. Molybdenum in soluble dietary forms is readily absorbed, and serum, whole-blood, liver, and kidney values reflect dietary intake (Herdt and Hoff 2011). Assessment of serum and hepatic molybdenum concentrations is useful as a reflection of potentially excessive intake with concomitant secondary copper deficiency. High serum molybdenum concentrations should cause concern for the presence of thiomolybdates, which could affect the interpretation of serum or plasma copper concentrations.

Selenium (Se)

Some of the physiological functions of selenium are still not clear, but much has been elucidated since the discovery of selenium as an integral part of cellular glutathione peroxidase enzymes (GPx). These enzymes prevent cellular damage by destroying hydrogen peroxide and lipid hydroperoxides. Selenium is also involved in the deiodination of thyroxine (T4) to the more metabolically active triiodothyronine (T3) in tissues. The immune system is adversely affected by selenium deficiency, and selenium deficiency increases the incidence of mastitis and retained placenta in dairy cows.

As an essential mineral, selenium is commonly identified as deficient in ruminants, but infrequently in dairy cattle. In dairy cattle, we see deficiency in dairy heifers and calves. Selenium deficiency is associated with reduced growth rates, poor feed efficiency, poor immune function, impaired reproductive performance, and damage to muscle tissue. "White muscle disease", or nutritional myopathy, is linked to severe selenium and/or vitamin E deficiency.

Cows will do all they can to ensure adequate selenium levels in calves when they are born. They will deplete their own body reserves to ensure neonatal adequacy. Therefore, a calf born with selenium deficiency confirms maternal

deficiency. This means that the dam will most likely have poor colostrum quality and inadequate immune protection for her calf.

Diagnosis of deficiency can be made by analysis of liver, whole blood, or serum for selenium, or by analysis of whole blood for glutathione peroxidase. Serum reflects the recent intake of selenium. Whole blood better reflects the longer term intake of selenium. In order to adequately diagnose selenium deficiency, the dietary form of selenium intake is important. The "adequate" concentrations of serum and whole blood selenium differ depending on whether the dietary selenium is in a natural organic form or an inorganic form.

Selenium excess is commonly identified in multiparous dairy cows. If the selenium excess is great enough, it can result in poor reproductive performance, poor calf survival, and imbalances of other minerals. Excessive selenium can also interfere with zinc absorption. The recommended adequate liver selenium concentration in adult cattle is 0.25 to 0.50 ppm. In comparison, late-term fetal or neonatal liver values should be higher, at 0.35 to 0.75 ppm.

Zinc (Zn)

Zinc is an essential component of over 70 enzymes found in mammalian tissues. Enzymes that require zinc are involved in protein, nucleic acid, carbohydrate, and lipid metabolism. Zinc is also important for normal development and functioning of the immune system, in cell membrane stability, and gene expression.

Responses of dairy cattle to zinc supplementation of practical diets have been highly variable, suggesting that dietary factors affect zinc bioavailability; however, these are not well defined. Some studies would suggest that high dietary calcium reduces zinc status in cattle (Spears, 2003).

Deficiencies of zinc are associated with reduced growth, poor immune function, diminished reproductive performance, and poor offspring viability, as well as skin lesions in severe cases. Liver and serum are the best indicators of zinc status. Response to zinc supplementation has shown that some animals with borderline zinc levels can still show improvement in some clinical conditions (Hall, 2010).

A number of laboratories have found decreasing zinc levels in multiparous cows over the last few years. They have also found excessive copper and selenium in the livers of these cows. The dietary excess of copper and selenium can interfere with zinc absorption; therefore, the low zinc levels are likely a secondary effect. It should be possible to decrease the copper and selenium levels in the rations to increase the absorption of zinc.

▪ Conclusions

A variety of sample types can be tested for trace mineral content, but may not provide an indication of overall mineral status of the animal. Diagnosis of trace mineral status involves evaluation of appropriate samples from groups of animals, rather than individuals. The evaluation should include a thorough health history, feeding history, supplementation history, and analysis of several animals for their mineral status.

Dietary mineral evaluation should only be used to augment the mineral evaluation of animal groups. If minerals are deemed to be adequate in the diet, but the animals are found to be deficient, antagonistic interactive effects of other minerals and true average daily per animal intake of the supplement need to be investigated. For example, high sulfur or iron causes deficiencies in copper and selenium, and excessive copper and selenium can adversely impact zinc status.

Common trace mineral deficiencies or excesses are significant hindrances to profitability in dairy cattle. They may impact reproductive performance, milk production, and animal health. In dairy operations, one must correctly identify the cause of the mineral imbalance, and any abnormal supplementation. We have seen cases with excessive supplementation in multiparous cows, but the replacement heifers were on a different ration and were deficient.

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Protein and Amino Acid Requirements of the Close-up Dry Cow

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▪ Take Home Messages

▸ Metabolic adaptations of maternal metabolism are essential to maintain nutrient availability in support of fetal development and lactation. Mobilization of body protein may be an underpinning issue leading to metabolic derangements and immune dysfunction and a greater risk of postpartum disease and impaired reproduction.

▸ Any process resulting in increased inflammation or excessive mobilization of body fat can lead to altered amino acid prioritization away from constituent protein metabolism resulting in exacerbation of protein mobilization and greater risk for disease, lost production or impaired reproduction.

▸ Close-up dry diet formulation needs to address meeting the rumen microbial population needs relative to energy and protein sources, which depends upon dietary forages and starch content, and the cow's additional needs to meet her amino acid requirements.

▸ Feeding higher amounts of metabolizable protein (MP) in dry diets may help to ensure adequate intake in the face of variable dry matter intake within a group. A concentration of 90–100 g MP/kg dry matter in the close-up diet is recommended to achieve at least 1100 g MP per day for a greater proportion of cows in a group.

▸ Research studies defining specific amino acid requirements in support of late pregnancy are limited and further research is required. A current body of research is suggesting methionine supplementation in late pregnancy may support improved health and immune status.

▪ Introduction

Dry cow nutrition and management more than 20 years ago was characterized as "management by neglect", a result of the lack of understanding we had on how nutritional management influenced all aspects of postpartum health, production and reproduction (Van Saun, 1991). Since the first description in the 1970's of "fat cow syndrome" and related energy-balance concerns, most dry cow research focused on energy metabolism and intake. Although higher protein supplementation to dry cows was suggested, subsequent research was equivocal in showing improved productive responses (Bell et al., 2000). Recent research is becoming more interested in transition cow protein needs based on documented body protein mobilization in support of early lactation (Grummer and Ordway, 2011). Anecdotal observations from the field would suggest dietary protein content, defined as metabolizable protein (MP), and possibly amino acid supply are having positive impacts on cow performance, but mainly from reproductive and metabolic health perspectives. The objective of this presentation is to address our current understanding of close-up dry cow protein requirements, provide perspective on transition protein metabolism and amino acid needs, and define practical feeding recommendations.

▪ Dry Cow Protein Requirement

Fetal growth from time of conception to birth can be described by an exponential growth curve with more than 70% of growth occurring in the last 60–70 days of pregnancy. This places the greatest nutritional burden of pregnancy on the close-up dry cow just weeks before parturition when there is potential for highly variable feed intake depending upon grouping strategies and feeding management.

Defining the Protein Requirement

The National Research Council (NRC) dairy and beef cattle publications over the past 60 years have defined and improved upon models to predict energy and protein requirements (Table 1) in support of pregnancy (NRC, 2001), though minimal differences are seen between reports due to a lack of data characterizing fetal protein requirements. Early NRC requirements were based on a 1950's extension publication and a 1956 study describing fetal growth in Danish Red cattle. The work of Bell et al. (1995) described growth characteristics for the modern day Holstein fetus and was incorporated into the most recent NRC model, though this model still did not totally account for all amino acid needs of the close-up dairy cow as it did not address mammary growth. The unknown factor in defining pregnancy protein needs is the amino acids needed to maintain labile protein reserves and their role in production, health and reproduction. All pregnancy requirement models are based on

research end points of milk yield or composition and do not address potential loss of body protein to support fetal requirements.

Table 1. Comparison of crude (CP) and metabolizable (MP) protein requirement models for a 650 kg mature cow at 270 days pregnant with a 45 kg birth weight calf.

	NRC 1989	NRC 2001	NRC 2001 Modified	CNCPS[a]/0.33
Maintenance				
Urinary, g/d	105	105	105	105
Scurf, g/d	15	15	15	15
MFN[b], g/d	410	338	338	338
Conceptus, g/d	212	355	355	480
Mammary, g/d	0	0	120 – 200	120-200
Total MP, g/d	742	813	933 – 1013	1058-1138
Crude Protein[c]	1060 g/d 8.2 – 9.6 %	1160 g/d 8.9 – 10.5 %	1332-1447 g/d 11.1 – 13.1 %	1511-1625 12.5 – 13.7%

[a] Cornell Net Carbohydrate and Protein System, ver. 3.0 with modification changing MP efficiency from 0.5 to 0.33
[b] Metabolic fecal nitrogen
[c] Assumed dry matter intake between 11 and 13 kg/d

Modeling Metabolizable Protein Requirement

Modeling gestational MP requirement is complicated as evidenced by model variation depicted in Figure 1. A proportion of the differences among these models is due to assumed efficiency of converting net protein (i.e., retained within the fetus) to MP (i.e., absorbed amino acids). Models prior to 1995 used an efficiency of 50%, whereas Bell (1995) summarized data suggesting efficiency was lower at 33%. This lower efficiency increases pregnancy MP requirement by 150%. Other challenges in predicting gestational protein requirements result from the dynamic metabolic functions of amino acids in supporting placental and uterine growth as well as the significant role amino acids play in fetal energy metabolism, none of which contribute to fetal protein retention, which is the measured end point. Another consideration is whether or not experimental diets were properly formulated to meet or exceed cow requirements to maintain a stable labile "reserve" protein pool in the cow. This is an underlying assumption of NRC models; maternal skeletal muscle is not used in support of pregnancy. McNeil et al. (1997) showed lamb birth weights were not different from ewes fed energy adequate diets with either 12% or 15% CP diets. Body compositional analysis, however, showed ewes fed the 12% CP diet (NRC requirement) had significant skeletal muscle protein loss accounting for the lack of difference in birth weights. Ewes fed the 15% CP diet had significant skeletal muscle accretion suggesting these ewes may be better positioned metabolically to adapt to negative energy balance and mobilize amino acids to support lactation. Could this situation account for the

greater rate of metabolic disease experienced by cows delivering twins in that the cow would mobilize her body protein to support the additional fetal mass with twins? Cows in the Bell et al. (1995) study consumed 10–12 kg dry matter of a total mixed ration (TMR) containing 13% and 14% (after 250 days gestation) CP. No measure of maternal protein status was determined in this study. So does the lower MP efficiency observed by Bell account for mobilization of maternal body protein?

For demonstration purposes we used the original Cornell Net Carbohydrate and Protein System (CNCPS, version 3.0) mechanistic model to predict gestational protein requirement, which accounted for an amino acid energy contribution, to predict MP requirement using an efficiency factor of 0.33 rather than the original 0.5 factor. From Figure 1 it can be seen this model greatly increases MP needs throughout gestation compared to other models. Additionally MP required to support mammary development (120-200 g MP/day) would need to be added to this model (Bell, 1995). More importantly this model shows MP needs before the 190 day cutoff used by NRC based on data extrapolation limitations. This exercise is hypothetical, but intriguing relative to potential implications for gestational MP requirements as well as explaining possible roles for amino acid nutritive status relative to health (i.e., immunologic and metabolic), productive, and reproductive outcomes during transition. This hypothetical model could potentially explain the observed positive cow responses in the field when additional protein is fed in close-up dry cow diets. Whether the response is due to higher protein requirement or meeting a specific amino acid need remains to be determined.
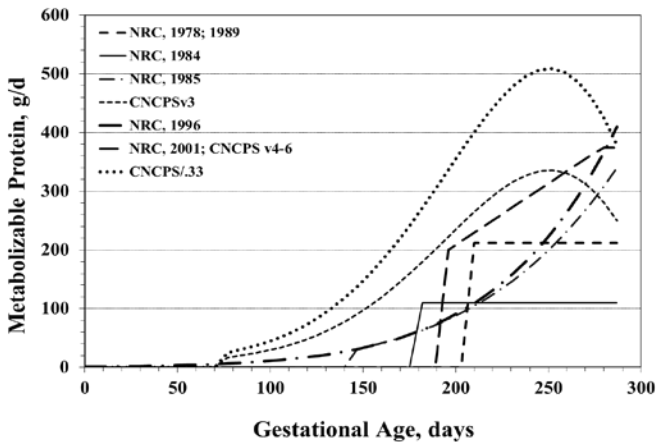

Figure 1. Different models predicting metabolizable protein (MP) requirement in support of pregnancy (45 kg birth weight) in Holstein cows (From Van Saun and Sniffen, 2014).

- # **Pregnancy Protein Metabolism**

Much emphasis has been placed on energy metabolism and markers of energy balance as underpinning metabolic disturbances of transition and risk for disease. Although elevated concentrations of either nonesterified fatty acids (NEFA) or β-hydroxybutyrate (BHB) are highly associated with disease risk, their presence is not an absolute determinant. A population of cows can perform without evidence of disease with elevated concentrations of NEFA suggesting some other factor or protective element. As our understanding of transition metabolism sheds more light on its complicated nature, a more integrated perspective on transition metabolism is needed and central to this is the supply and prioritization of amino acid metabolism as it relates to cow response to diet and management. Although the body of published literature does not strongly suggest improved cow performance with greater prepartum dietary protein, there is much interest and anecdotal observations suggesting benefits from feeding diets delivering greater MP (>1100 g/day) than models would suggest is necessary to meet the cow's amino acid requirements. This observed response may be due to an underestimation of the MP requirement, providing an essential amino acid or acids, accounting for intake variability within a group allowing for adequate MP intake for cows with lower intake, or some combination of these factors.

Most studies evaluating prepartum protein nutrition essentially looked at milk yield or composition as metrics for a measured response (Bell et al., 2000). Most observations and research would suggest the primary benefit of prepartum protein feeding comes from disease prevention and improved reproductive performance. Curtis et al. (1985) reported higher prepartum protein diets decreased incidence of ketosis. Van Saun (1993) also reported lower clinical ketosis prevalence for mature Holstein cows fed 1350 g MP/day compared to cows fed 1100 g/d. In this study, all cows maintained a higher body condition score (mean 3.9 at calving), thus were more predisposed to ketosis problems. Using 3-methylhistidine as a marker of skeletal muscle degradation, van der Drift et al. (2012) showed muscle mobilization occurring prepartum through 4 weeks postpartum for dry cows fed a diet composed of grass silage and corn silage containing approximately 12.6% crude protein. Cows having higher 3-methylhistidine concentrations generally had lower BHB concentrations, suggesting a protective effect. Cows with extreme hyperketonemia had excessive muscle and fat mobilization, which could be detrimental to health and reproduction. Philips et al. (2003) showed supplementing methionine prepartum may mitigate body protein mobilization, possibly suggesting a higher amino acid requirement.

Mobilized protein from skeletal muscle and involuting uterine tissue provides a primary source of amino acids to the mammary gland to support milk protein synthesis. Lower milk protein content may reflect inadequate dietary MP

supply and repartitioning of amino acids to support the immune response or gluconeogenesis. In reviewing lactation performance across many herds, cows with low milk true protein (<2.7%) on first or second test day had lower first service and overall conception risks. Cows consuming more MP prepartum (>1350 g/d) had improved reproductive performance, and ovulation time was not influenced by negative energy balance nadir. In contrast, cows consuming lower prepartum MP intake (1100 g/d) followed by a postpartum diet high in RDP had their first ovulation time highly correlated with negative energy balance nadir (Van Saun, 1993). Availability of amino acids may be a critical factor in early follicular development and ultimately conception risk.

Unfortunately there is no single simple blood parameter that reflects protein status such as NEFA or BHB relative to energy status. Blood albumin concentration has been used as a proxy for protein status. Albumin concentration reflects dietary amino acid supply and metabolic responses repartitioning available amino acids. Increasing dietary protein in early lactation increased albumin concentration. Albumin is synthesized in the liver and is considered a negative acute phase protein meaning its rate of synthesis is decreased during an acute phase response to inflammatory cytokines (Bertoni et al., 2008). Albumin concentration pre- and postpartum was associated with greater risk for postpartum disease. Blood albumin concentration ≥35 g/L was found in primarily healthy fresh cows compared to lower concentrations being predominately associated with fresh cows having one or more disease events. Lower albumin concentration may reflect inadequate dietary MP supply, liver dysfunction, an active inflammatory response, or some combination, and may provide a marker of transition cow health status (Overton and Burhans, 2013).

It is our assessment that amino acids play a critical role in "stabilizing" metabolism of carbohydrates and lipids during transition as well as supplying substrate for tissue protein synthesis, gluconeogenesis, and other metabolic mediators. All cows experience a period of negative protein balance in early lactation that seems somewhat independent of prepartum protein feeding. If dietary protein is sufficiently deficient prepartum, however, tissue protein mobilization may occur and the reservoir of labile protein to be utilized in early lactation may be compromised resulting in greater risk for impaired health, productive efficiency, and reproductive performance (Ji and Dann, 2013).

Role of Inflammation on Protein Metabolism

A growing body of research is recognizing an association between the activated inflammatory response mediated by pro-inflammatory cytokines interleukin (IL)-1, IL-6, and Tumor Necrosis Factor (TNF)-α and altered metabolism leading to greater disease risk, poor production, and impaired reproduction (Bertoni et al., 2008; Bradford, 2015). Pro-inflammatory

cytokines can be released from adipose tissue during mobilization as well as from any stress response. Hepatic activation by these cytokines initiates the acute phase protein response resulting in up-regulated synthesis of positive acute phase proteins (+APP; i.e., ceruloplasmin, haptoglobin, serum amyloid-A, C-reactive protein, complement components) as well as enzymes and other physiologic mediators. Both IL-1 and TNF-α have profound metabolic effects promoting an increased basal metabolic rate to produce fever in concert with reducing appetite. Reduced appetite in the transition cow is a recognized lynchpin to metabolic disease susceptibility. Mobilized skeletal muscle provides amino acids to support gluconeogenesis in maintaining the higher basal metabolic rate. This response is in an effort to promote the immune response in responding to some pathogen or stressor, but is quite costly nutritionally to the animal.

Mobilization of skeletal muscle will further exacerbate negative protein balance in early lactation and may account for the predilection for more than one disease process once one has been established (Ji and Dann, 2013). In addition to mobilization of skeletal muscle, constitutive proteins synthesized by the liver, such as albumin, retinol binding protein, apoproteins, and transferrin (e.g., negative acute phase proteins, -APP) are not synthesized, most likely to further provide amino acids to support the acute phase protein response (Bertoni et al., 2008). Reduction of these constitutive proteins may adversely affect mineral and vitamin metabolism through the loss of transport proteins. Additionally, loss of apoproteins would reduce the liver's ability to synthesize very low density lipoproteins and potentially increase fatty infiltration in the face of elevated NEFA concentrations. An activated immune response is necessary during transition to deal with uterine clearance and protection from potential mastitis pathogens, but excessive stimulation of this response through environmental, social, or dietary factors will predispose to poor transition cow performance.

▪ Amino Acid Requirements and Supply

As our understanding of nutrient requirements increases there is a natural evolution of defining "protein" requirements from nitrogen-based crude protein (i.e., N x 6.25) to MP to finally defining specific amino acids, which are the actual substrates needed by the cow. Poultry and swine nutritionists have made this progression in protein requirements where they formulate for specific amino acids to achieve the "ideal protein" in the diet. At this point in dairy cattle requirements we have not defined specific amino acid requirements.

A body of research is focusing on supplemental methionine, an essential amino acid, as a critical amino acid for transition cows. The work of the Illinois team has placed emphasis on the requirement for methionine in the close-up ration. Their results have demonstrated that there is a need relative to lipid

mobilization and immune function, which has resulted in production responses (Osorio et al., 2014). The question one needs to ask is: are there essential amino acids required beyond methionine, such as the branch chain amino acids, histidine, arginine and proline (Phillips et. al., 2003)? There is evidence from work done that lysine may be essential as well. Summarized studies using milk protein yield or percent as an endpoint suggest requirements of 25 g/d and 75 g/d for methionine and lysine, respectively (French, 2012). Further, should we think beyond this to consider the gluconeogenic amino acids as well? Bell (1995) has shown that there could be a significant requirement for gluconeogenic amino acids to meet the mammary, placental and fetal requirements for energy during the late gestation period. Additionally, Bell (1995) points out there is a significant increase in the requirement for hepatic protein synthesis, which in the last 2 weeks before birth is accelerated. The acceleration is driven by increased mammary requirements, increased demand for liver size, and the high requirements of nutrient fluxes through the liver to deal with increased metabolic requirements. This becomes particularly critical as we reduce the energy provided during both early and the close-up period, which will reduce the supply of propionate but also reduces microbial yield that provides essential amino acids as well as the non-essential amino acids. Larsen and Kristensen (2012) looked at amino acid net fluxes using arterial-venous differences coupled with blood flow, prepartum and postpartum using glucogenic and ketogenic diets. They demonstrated net negative hepatic fluxes prepartum of non-essential amino acids, lending credence to the importance of adequate non-essential AA as well as essential AA. Recent work by Penn State has shown histidine to be limiting in diets where rumen microbial growth accounts for the majority of MP needs (Lee et al., 2012). Most amino acid work has focused on milk yield or composition and during early to mid-lactation. Whether or not the dry dairy cow fits into the models predicting amino acid flow remains to be seen. Some of the current study analyses would suggest amino acid content of prepartum and postpartum diets are not independent of each other and one cannot make up for prepartum deficiencies with a better balanced postpartum diet. A recent study infusing casein into early lactation cows has shown the critical importance of MP, especially essential amino acids, in supporting milk yield and composition as well as immune function (Larsen et al., 2014). The protein effect was over and above any energy deficiency.

▪ Meeting the "Protein" Requirements

Dry cow protein nutrition has been misunderstood and is still a somewhat unknown area of investigation. Controlled studies in this area have many times been confounded by the method of balancing to meet the pregnant cow protein requirement. The NRC recommendations for protein supply were based on research that unfortunately was limited and experimental rations were often formulated inappropriately providing wrong conclusions. Further,

the recommendations did not recognize the importance of the mammary requirement and protein reserves. The CNCPS system now recognizes the importance of both; however, it does not recognize the importance of labile protein reserves relative to immune function as well as the need in the early postpartum period when cows can mobilize 800 to 1000 g/day (Bell et al., 2000). This puts greater emphasis on the maintenance of labile protein reserves in the last 60 to 80 days of gestation. This is a period in late lactation and during the dry period when lower energy rations are being fed, reducing microbial protein output and MP balance can easily become negative, especially with hay-crop silage based diets. Field observations would suggest there is a need to exceed the NRC (2001) recommendations for protein and meet and not exceed the ME requirements. Coupling this with variation in dry matter intake (DMI) within a group of cows being fed a balanced ration, dictates that there be an adequate concentration of MP in the rations being fed during this time in order to ensure that all cows will be able to maintain the protein reserves that were replenished in mid-lactation. Additionally recent work has suggested that protein quality may be important as well. This would suggest it is important to pay attention to source as well as amount of MP.

With current understanding of dairy cow feeding, we need to consider the close-up dry cow diet formulation process in two stages: 1. feeding the rumen to generate microbial mass, a significant contributor to MP, and 2. feeding the cow over and above what nutrients are not provided by rumen outflow. This approach is no different than how we formulate lactating cow diets. The only issue here is whether or not the dry cow rumen dynamics fit the predictive model between dietary fermentable energy and microbial growth. Microbial growth is dictated by availability of fermentable carbohydrate and with the growing application of low energy diets does this suggest lesser microbial growth and greater need for bypass protein sources to meet the cow's amino acid needs (Kokkonen, 2014)? Rumen fiber fermentation is dependent upon availability of rumen degradable protein, thus a minimum dietary protein content of 11-12% is needed to ensure microbial fiber degradation, which is above what NRC requirements would suggest for the dry cow diet protein content (Dorshorst and Grummer, 2002).

Accurately Defining the Cow

The definition of dry cow requirements is based on carefully defining first the dry cow group that is to be fed. We often get into the mode of using one set of numbers. This is inappropriate. The impetus of defining the animal correctly is to ensure the diet will provide sufficient nutrients to all individuals within the group. The question is whether the description should be the average for the group or the upper level? For example, if average calf birth weight is 42 kg, what happens to those cows delivering a 45 or 48 kg calf? Expected birth weight can significantly influence nutritional requirements. The biggest

challenge occurs in those mixed groups of springing heifers and mature cows. Obviously some animals will be overfed, but we want to minimize the underfeeding variation.

Ensuring Adequate Nutrient Intake

One of the primary challenges of dry cow group management is formulating the diet for an appropriate intake level. Even if one provides a balanced diet for a defined average intake for a given feeding group, 50% of the animals in the group consume less than the average intake. French (2012) presented summarized prepartum intake data from Phillips et al. (2003) for multiparous Holstein cows. In this analysis the average DMI was 12.3 ± 2.5 kg/d for the last 21 days precalving with 15% of the cows consuming less than 10 kg/d (1 standard deviation below group average) and being in a state of negative nutrient balance. A recommendation from this analysis was to formulate the close-up dry diet to 1300 g or 1400 g MP as a safety factor to ensure adequate numbers of cows, 83% or 95%, respectively, consume a desired 1,080 g MP from the diet.

In another multiparous cow dataset, 21 day prepartum DMI was 13.5 ± 2.6 kg/d (Van Saun, 1993). In this study, prepartum diets differed in MP content (1100 vs. 1350 g/d) but DMI was not different across treatments. The cows consuming the higher MP diet had less metabolic disease and improved reproductive performance compared to the lower MP diet. These results would seemingly support the concept promoted by French, though a higher MP requirement is not out of consideration in explaining such responses. Clearly, large variation (higher standard deviation) of DMI within a group will result in more cows, and especially heifers in mixed groups, having lower intake and potentially experiencing a negative MP balance. These two datasets would suggest formulating a close-up diet to contain between 90 and 100 g MP/kg dry matter, which would provide at least 1000-1100 g MP for those lower intake cows within the group.

▪ Conclusions

Observational performance on farms would suggest protein content and source in the close-up dry cow diet is a critical factor in ensuring cows transition smoothly into lactation and have good health with unimpaired productive and reproductive performance. We still have gaps in our understanding of amino acid metabolism and requirements in late pregnancy and how this may be influenced by diet composition, namely carbohydrate fractions. Improved descriptions of close-up dry cows relative to expected calf birth weight, body weight and condition score as well as accounting for parity differences can improve our dietary formulations for an optimum MP requirement. Adjusting dietary MP content to account for variability in group

feed intake is a critical factor in ensuring a greater majority of the individuals within the group will consume a minimum of 1,000 g MP/day. We recommend formulating the close-up dry cow diet to contain 90-100 g MP/kg to meet MP needs of the greater proportion of the group.

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Ovarian Activity Preceding First Insemination Affects Fertility in Postpartum Dairy Cows

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The high incidence of ovarian abnormalities early postpartum and embryonic losses are recognized problems, yet poorly characterized. To investigate if ovarian activity preceding first breeding postpartum could affect fertility, progesterone (P4) concentrations of 420 Holstein cows were determined through in-line milk analysis system from two herds in Alberta. Data were analyzed to estimate interval from calving to first ovulation (1stOv), number of cycles preceding 1stAI and outcomes (open, pregnant or pregnancy loss). 1stOv occurring ≤45 d was defined as Early (Early-Ov) and >45 d as Late (Late-Ov), and cycles were considered as normal (P4 >5ng/mL for 7 to 19 d) or abnormal (P4 >5ng/mL for <7 or >19 d). After AI, if P4 reached 5ng/mL (threshold) and remained higher high for more than 40 d, it was considered pregnancy. If P4 dropped below threshold between 20 to 40 d, pregnancy loss was considered. Overall, 33.4 and 26.2% cows were pregnant, and 13.7 and 12.4% had pregnancy loss after 1st and 2ndAI, respectively. 1stOv occurred later in primiparous cows than in multiparous cows (51 vs 47 d, p<0.01). Although, primiparous cows were 1.7 times more likely to conceive to 1stAI (p<0.05), they were 1.7 times less likely to conceive after 2ndAI compared to multiparous cows (p=0.05). Early-Ov cows were twice more likely to become pregnant to 1stAI than Late-Ov (p<0.01). Pregnant cows (and cows that had pregnancy loss) had earlier 1stOv (38.7 d) than open cows (45.6 d, p<0.01) and had more normal and total cycles before 1stAI than open cows (1.16 and 1.67 vs 0.87 and 1.43, respectively; p<0.05). However, the prevalence of abnormal cycles preceding 1stAI was not different between cows that remained open, became pregnant or that lost pregnancy.

Take Home Message: Cows ovulating earlier postpartum, had a greater chance of conceiving to 1stAI. However, primiparous cows were more fertile than multiparous cows even though they had a longer interval from calving to 1stOv. Cows that conceived to 1stAI had more ovarian activity preceding that AI than cows that did not conceive. Ovarian activity preceding 1st AI did not differ between cows that successfully maintained the pregnancy and cows that underwent pregnancy loss.

The in vitro Inhibitory Effect of Coagulase-negative staphylococci on Major Mastitis Pathogens

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The most commonly isolated bacteria from the udder are coagulase-negative staphylococci (CNS), a group of approx. 50 species. Several studies have found that CNS have a protective effect against infection of the udder by major mastitis pathogens, while other studies have reported no protection. This inconsistency is likely due to conflicting effects by different CNS species that were earlier undifferentiated. The first aim of this study was to characterize CNS species-specific inhibitory effects against major-mastitis causing pathogens. The second aim was to characterize the inhibitory compound being produced by the CNS, and then to identify candidate genes with known bacteriocin or immunity function in whole genome sequences obtained from isolates. We hypothesize that some species will display inhibitory capabilities against major pathogens and that the inhibitory product is a bacteriocin, with genes present in various isolates and species of CNS.

Materials and Methods: The two projects used CNS and pathogen isolates obtained from the Mastitis Pathogen Collection of the Canadian Bovine Mastitis and Milk Quality Research Network. Species-specific inhibition was tested for using a modified cross-streaking method from De Vliegher et al. (2004). Of the 39 CNS isolates tested, 9 isolates, comprised of 3 *Staphylococcus chromogenes*, one *S. simulans*, one *S. epidermidis*, one *S. sciuri*, and 3, inhibited growth of Gram-positive bacteria, such as *Staphylococcus aureus*. Characterizing and identifying the compound is currently underway and will be done using a well-diffusion assay, liquid chromatography, mass spectrometry, and whole genome sequencing. We will identify which isolates possess bacteriocin-related genes and examine how common they are in an addition 500 CNS isolates of which we have a whole genome sequence.

Implications: Determining species-specific effects will allow better understanding of how CNS infections affect udder health. Identifying bacteriocins may lead to novel antimicrobials to be used for the prevention and treatment of Gram-positive pathogens, which will improve udder health and decrease the economic impact of mastitis.