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Nutraceuticals as an Alternative Strategy for the use of Antimicrobials

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■ Take Home Messages

- ▶ Nutraceutical is a term derived from nutrition and pharmaceutical. There are many compounds that improve immune responses and reduce the risk of disease through different mechanisms of action.
- ▶ Prebiotics (biological modifying polysaccharides) are a group of indigestible carbohydrates that improve the growth of commensal bacteria. Some fractions (e.g., β -glucans and mannan-oligosaccharides) have direct immunomodulatory, gram-negative pathogen binding, or hydrophobic mycotoxin absorption capacities.
- ▶ Probiotics (direct-fed microbials) are viable microorganisms supplemented to an animal that offer potential health benefits to the animal. Probiotics are predominately supplemented to improve gastrointestinal health, rumen fermentation, or nutrient utilization.
- ▶ Phytonutrients are a group of compounds, isolated from plants, with potential therapeutic applications because of their intrinsic antioxidative, anti-inflammatory, and antimicrobial properties.

■ Introduction

Zoonotic multi-drug resistant bacterial strains and antibiotic resistance have encouraged changes to on-farm antimicrobial use. Veterinarians and producers are evaluating alternatives to the use of antimicrobials. Nutraceuticals, primarily derived from microbial and plant-based compounds, are receiving increased research and commercial attention. Nutraceuticals are a diverse group of compounds and microbes that offer some advantageous effects to health and productivity, including improved feed efficiency or reduced disease through either immune modulation or decreased infection. A more extensive review on the topic is available at Ballou et al. (2019). Nutraceuticals can be classified in many ways, with the most common based on the mechanism of action, chemical structure, or the source of the compounds. In this paper I will focus predominately on the mechanism of action and will discuss three broad classes of nutraceuticals: biological modifying polysaccharides (prebiotics), direct-fed microbials (probiotics), and phytonutrients. Nutraceuticals are not regulated by the Food and Drug Administration; therefore, statements regarding composition, dosage, effectiveness, and quality are not independently validated or standardized. This makes comparison and interpretation among extracts and commercial products difficult.

■ Biological Modifying Polysaccharides

Indigestible carbohydrates, including oligosaccharides and fructans, can improve health through several mechanisms of action. One of these mechanisms is through a symbiotic or prebiotic effect, where these indigestible carbohydrates are an energy source for probiotic bacteria, and the health benefits are through improving the microbial ecology of the host gastro-intestinal tract. However, the focus of these indigestible carbohydrates in the current presentation will be on their immunomodulatory effects and ability to adsorb gram-negative bacteria as well as certain bacterial and fungal toxins.

Biological modifying polysaccharides are found in a variety of plants, milk, and the cell wall of fungi. I will focus on the polysaccharides extracted from cell walls of fungi, including mannan-oligosaccharides (MOS) and β -glucans (BG). The composition, availability, and physical chemistry of the extracts and carbohydrates influence their ability to improve the health of livestock. For example, a fungal extract from one fungus may behave very differently from another fungal extract. Further, many extracts are blended with other ingredients; therefore, one gram of Product A may have a different function than one gram of Product B. Unfortunately, the analytical chemistry to determine these structures and concentrations is complicated and expensive.

Type 1 fimbriae are mannose-specific filaments on the surface of pathogenic gram-negative bacteria. Ganner et al. (2013) conducted a study in which the fimbriae on gram-negative bacteria were adsorbed by yeast cell wall extracts, and the ability to adsorb both *Salmonella* species and *E. coli* were correlated with the concentration of MOS. The fimbriae adsorb to MOS and prevent the pathogenic bacteria from binding to the epithelium and colonizing the gastro-intestinal tract. Therefore, supplementing yeast extracts with greater quantities of MOS can be a useful prevention strategy for those animals with a high exposure to gram-negative pathogenic bacteria (Davis, 2018). The impacts of MOS on specific immune responses is not well understood. Further, most fungal extracts that contain MOS also contain BG, which are known to have immunomodulatory effects.

B-glucans from cell wall fractions from fungal sources have the potential for immunomodulatory effects. The β -1,3 glucans are able to ligate Dectin-1 receptors on monocytes, macrophages, neutrophils and to a lesser extent on dendritic cells and T cell surfaces (Taylor et al., 2002). Further, the size of the BG extract influences both the leukocyte type and the response that is impacted (Elder et al., 2017). Data indicate that smaller BG, which are more common on less virulent fungi, may limit inflammation, whereas large BG oligosaccharides may increase inflammation. Oral supplementation of BG may also impact systemic immune responses in addition to local gastro-intestinal immune responses. The systemic effects could be both direct and indirect. The direct systemic effect is thought to be mediated by M-cells, which are specialized cells that sample intestinal lumen contents, including BG. Preliminary data from our laboratory indicate that dairy calves supplemented with a BG extract had increased relative abundance of larger oligosaccharides, 7 and 8 oligosaccharides, in peripheral circulation when compared to calves not supplemented BG (Davis and Ballou, unpublished).

■ Direct Fed Microbials

Direct-fed microbials, also known as probiotics, are live microorganisms that can improve the health and performance of livestock. Common commercially available microorganisms include *Lactobacillus* species and other lactic acid-producing bacteria, *Bifidobacterium* species, *Bacillus* species, and *Saccharomyces cerevisiae*. The dose is commonly reported as colony forming units supplemented per day or per kg of dry matter. Other important considerations when supplementing direct-fed microbials include the age or physiological state of the animal, infectious pressure, and the duration of supplementation. Oral supplementation of direct-fed microbials to impact gastro-intestinal health makes the most sense because the supplemented microorganisms target the microbial communities and cellular function within the gastro-intestinal tract.

The gastro-intestinal tract is a dynamic tissue that varies between animals, diets, age, environment, and management factors. To be considered a microorganism with probiotic effects, the microorganism should provide at least one of the following desirable outcomes:

- Regulate gastro-intestinal tract microbial communities
- Prevent adherence of potential pathogens in the gastro-intestinal tract
- Product anti-microbial or bactericidal molecules
- Improve gastro-intestinal tract integrity
- Improve mucosal adaptive immune responses
- Balance gastro-intestinal inflammation
- Improve fermentation and nutrient utilization

The application of direct-fed microbials in neonates is common. The gastro-intestinal tract of these animals is rapidly developing, and these animals are more susceptible to gastro-intestinal disease. Early in life the gastro-intestinal tract is colonized with facultative anaerobes (microorganisms that can survive with or without oxygen), which includes many bacteria from the environment, and then shifts more toward strict anaerobes (microorganisms that die in the presence of oxygen; Meale et al., 2017). Therefore, supplementing anaerobic lactic acid producing bacteria may speed up the microbial progression and reduce the risk for infection from environmental Enterobacteriaceae (Liang et al., 2020). The model of competitive exclusions has been around for a long time, where beneficial microorganisms take up space and use nutrients that are then less available for disease-causing microorganisms. Further, lactic acid producing bacteria can help lower pH in the lumen of the gastro-intestinal tract, which can help limit the establishment of pathogenic Enterobacteriaceae.

Another mechanism through which direct-fed microbials can improve gastro-intestinal health is by modulating the gut-associated mucosal tissue immune system. Many immune factors, including secretory IgA, antimicrobial peptides, and other regulatory leukocyte responses, concentrate themselves locally in the gastro-intestinal mucosa. The immune factors are important to maintain gastro-intestinal integrity and function, as well as balance the local inflammatory response. Liang et al. (2020) conducted a study with two groups of Jersey bull calves: one group supplemented with a blend of two strains of lactic acid producing bacteria and one group not supplemented. Both groups were then challenged with a moderate dose of *Salmonella typhimurium*. The calves that were supplemented with the direct-fed microbials had reduced systemic and local inflammation after they were infected with the *Salmonella typhimurium*. Localized inflammatory responses are often considered beneficial in most tissues; however, in the gastro-intestinal tract an excessive or prolonged inflammatory response can further exacerbate the pathogenesis of the disease because of impaired gastro-intestinal integrity.

Direct-fed microbials in adult livestock are also used to support gastro-intestinal health. However, in adult ruminants many of the direct-fed microbials are fed to target the rumen and improve nutrient digestibility and utilization. Although the main target is the rumen, some of the direct-fed microbials can make their way through the rumen and have similar impacts on intestinal health as noted above for young calves. In fact, supplementing direct-fed microbials to feedlot cattle is a common industry practice for preharvest food safety. Cattle supplemented with direct-fed microbials had decreased fecal shedding of pathogenic bacteria and decreased carcass contamination from the same bacteria (Brashears et al., 2003; Younts-Dahl et al., 2005; Peterson et al., 2007). In lactating dairy cows, most of the data focuses on production performance and milk quality. Fecal pathogen shedding or manure consistency are not often reported, but conceivably some of the performance benefits may be partially attributable to improvements in gastro-intestinal health. Further, the greatest health and economic benefits of supplementing direct-fed microbials are during stressful events, such as the transition period.

■ Phytonutrients

Phytonutrients are a broad group of compounds with potential therapeutic applications because they have antioxidative and anti-inflammatory properties. Plants synthesize polyphenols as a defense mechanism against both potential pathogens and ultraviolet irradiation. Various fruit and vegetable byproducts that are rich in phenolic compounds are available as feedstuffs for ruminants and may include citrus, grape, pomegranate, and green vegetable processing residues. Polyphenolic compounds can be absorbed in the small intestines and enter peripheral circulation where they can exert their bioactive effects on various tissues. The ruminal environment can modulate the activity of the dietary polyphenols. The rumen microbial communities can degrade polyphenols and decrease host availability. Therefore, the biological activity will depend on the structure in the diet as well as the concentration of the bioactive ingredients that bypass through the rumen.

Published reports on the immune effects of feeding flavonoid-rich products to ruminants are limited to predominately grape, pomegranate, and green tea derivatives. Supplementing grape polyphenols lowered oxidative damage in postpartum dairy cows (Colitti et al., 2006). Dairy calves supplemented with a pomegranate extract had increased measures of immune function including, *in vitro* secretion of interferon- γ and interleukin-4 as well as greater ovalbumin specific immunoglobulin G responses (Oliveira et al., 2010). Lastly, polyphenols from green tea extracts reduced the inflammation of small ruminants following a parasitic challenge (Zhong et al., 2014). These data suggest that the antioxidative and anti-inflammatory properties of these polyphenolic-rich compounds can play a role in improving the health of livestock.

Essential oils are another class of phytonutrients. In addition to immunomodulatory effects, some essential oils have antimicrobial activity against food borne pathogens and rumen microorganisms. The majority of research on the immunomodulatory effects of essential oils was conducted in monogastrics. Some of the mechanisms of action are through a direct receptor-mediated improvement in mucosal blood flow, altered cytokine and neuropeptide release, or modified leukocyte function. In ruminants the main essential oils investigated were carvacrol and thymol from oregano oil, garlic, and capsaicinoids, but due to ruminal degradation the impacts of some of these supplements in mature ruminants are not well understood. Supplementing milk-fed dairy calves with oregano oil reduced the incidence of scours, improved hematology, and increased immunoglobulin concentrations of the calves (Katsoulos et al., 2017; Seirafy and Sobhanirad, 2017; Ozkava et al., 2018). However, in lactating cows topical or intramammary administration of oregano failed to cure an experimentally-induced *Streptococcus uberis* infection. However, intra-abomasal infusion of garlic oil increased the neutrophil to lymphocyte ratio as well as increased the CD4 positive T cell population, and similarly, intra-abomasal infusion of capsaicinoids increased CD4 positive T cell proliferation (Oh et al., 2013). Lastly, capsaicinoids were able to reduce the acute phase response to an intravenous lipopolysaccharide challenge in mature dairy cows (Oh et al., 2015).

■ Summary

Nutraceuticals are a diverse group of compounds. To be considered a nutraceutical the oral supplementation must improve some aspect of animal health or production efficiency. There remains a lot of ambiguity regarding nutraceuticals because this is a rapidly evolving field without a lot of regulatory oversight. The concentration of bioactive ingredients or compounds is often not known or reported. Further, a lot of commercial products are extracts that can contain many different bioactive compounds that may work in a symbiotic or opposing manner. The presentation discussed nutraceuticals as biological modifying polysaccharides, direct-fed microbials, and phytonutrients. These compounds work through a variety of mechanisms including stabilizing microbial communities, improving mucosal responses and barrier function, adsorbing potential pathogens or toxins, improving antioxidant status, direct antimicrobial activity, and either increasing or decreasing systemic leukocyte responses.

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Protein During the Dairy Cow Transition Period: What We Feed and What the Cows Lose

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■ Take Home Messages

In late gestation and early lactation there are drastic increases in the amino acid (AA) requirements of dairy cows to meet increasing fetal and milk production requirements. Extensive research has shown potential benefits to supplementing additional metabolizable protein (MP) and specific rumen-protected AA in late gestation including improved production, immune status, and reproductive success. Variation exists for supplementing rumen-protected AA in research trials and on commercial herds likely because of interactions between AA and other nutrients supplied. To meet requirements in early lactation, cows mobilize both adipose tissue (fat) and muscle to meet energy and AA requirements. The extent that dairy cattle lose adipose tissue is well documented with negative health outcomes being associated with excessive adipose tissue mobilization; however, protein mobilization is less well understood. In high producing dairy cows, there is a considerable negative MP balance in early lactation that has to be met by mobilized skeletal muscle. A portion of the skeletal muscle can be mobilized but there appears to be a minimum amount of empty body protein that must be maintained. Considerable variation exists between the amount of muscle depth at specific locations of the body that is representative of whole body muscle. There is a moderate negative relationship between the amount of muscle depth prepartum and the extent of muscle that is mobilized through early lactation. On average, cows mobilize approximately 20% of their muscle depth from calving through 30 days in milk (DIM). However, there is considerable variation among cows; these differences in metabolic efficiencies need to be explored. Nutritional considerations for the extent of tissue that is mobilized need to be considered in order to truly maximize production, reproduction, and health outcomes.

■ Introduction

In the transition from late gestation to early lactation cows undergo considerable metabolic adaptation to support demands for fetal growth, parturition, and upcoming milk production (Bauman and Currie, 1980). The dairy cow has three main stores of tissue she can mobilize to meet increased requirements: glycogen, adipose tissue, and muscle. Considerable metabolic adaptation occurs in muscle; increased protein catabolism and reduced protein anabolism occur during the transition period. Increased proteolysis (muscle breakdown) contributes AA to the developing fetus in late gestation and provides AA to support colostrum and milk production in early lactation. This increase in proteolysis is a normal homeostatic response of animals adjusting to a new normal where their demands have changed without a sufficient increase in intake to support the increased requirements. Supplementing rumen-protected AA during the transition period has the potential benefit of increasing MP and individual AA that may be limiting in the transition cow.

The dairy industry is more familiar with measuring the amount of adipose tissue than the amount of muscle that cows mobilize around the transition period. By using body condition score (BCS) we can visualize changes in subcutaneous fat thickness that occur around calving. We can also measure metabolites related to adipose tissue mobilization such as non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHB). While change in BCS is generally associated with changes in adipose tissue, it is moderately correlated to changes in empty body protein as well (NRC, 2001). For the transition dairy cow, depending on BCS, adipose tissue may contribute to over 25% of the empty body weight (BW) of the cow, with protein

representing approximately 13% of empty BW (Komaragiri and Erdman, 1997). Metabolic adaptation to early lactation may result in mobilization of > 80 kg of adipose tissue and 20 kg of protein. From a research perspective we can evaluate muscle mobilization by using ultrasounds and assessing metabolites including creatinine and 3-methylhistidine; however, these are not routinely done on commercial farms. Farms that are implementing several BW measurements during the transition period are assessing whole body changes that include both adipose and muscle tissue changes. Commercial farms may use these changes in BW to determine the relative extent of negative energy and MP balance and may make different breeding strategies based on the extent of tissue lost, with cows that lose more weight having extended voluntary waiting periods compared with cows that lose modest amounts of weight. While BW is not specific for tissue type it can help producers identify individual animals with more severe negative energy and MP balance during the transition period.

■ **Amino Acid Requirements for Dairy Cows During the Transition Period**

Because of the inability of dairy cows to consume sufficient dry matter to meet nutrient requirements during the transition period, cattle are more susceptible to metabolic diseases during this time. The ability of the dairy cow to adapt to changing demands of late gestation into early lactation dictates her ability to have a successful lactation for milk production, reproduction, and health outcomes. Changes in insulin sensitivity in early lactation allow for an increased amount of nutrients to be partitioned to the mammary gland, potentially exacerbating the negative effects of negative energy and MP balances.

Cows will meet their AA requirements through MP from feed and microbial protein and from catabolism of skeletal muscle (Figure 1). These AA are then used for several outcomes including fetal AA requirements, milk protein synthesis, and the production of ketones or glucose depending on stage of gestation and lactation. The current NRC (2001) model suggests that in late gestation, Holstein dry cows should receive 850 g/d of MP whereas most nutritional models would suggest MP supply in excess of 1,200 g/d may be more appropriate (recommendations will vary depending on nutritional model used). During the close-up dry period, the MP requirements for the uterus and fetus increase considerably (Bell et al., 1995). Additionally, there are considerable increases in MP requirements for mammary gland development and colostrum synthesis in the last weeks of gestation (Capuco et al., 1997; Bell et al., 2000). As BW and milk production for dairy cattle have increased and our understanding of the role of individual AA has increased, most nutritionists would recommend providing MP in late gestation compared with that recommended by NRC (2001).

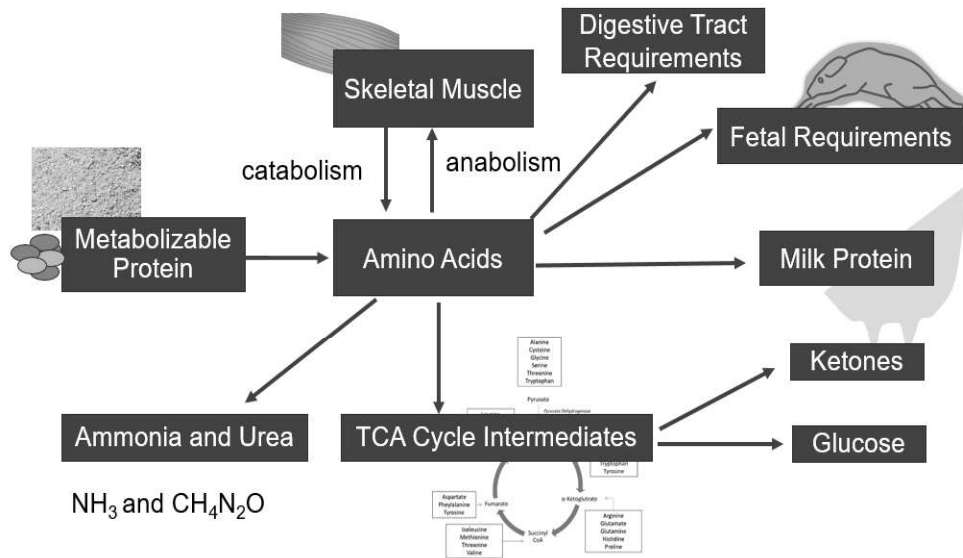


Figure 1. Sources and uses of amino acids in the transition dairy cow.

In early lactation, the requirement for MP significantly increases as milk production and milk protein yield increase. Additionally, MP is used for increases in the mass of the digestive tract and liver, which increase in size to increase absorption of nutrients and production of glucose required in early lactation (Reynolds et al., 2004; Aschenbach et al., 2010). Bell et al. (2000) suggested that cows are in negative MP balance until approximately 21 days postpartum because of increased requirements in early lactation relative to insufficient dry matter intake. At its nadir, MP balance may be in excess of -600 g/d, with the balance needing to come from skeletal muscle mobilization. The severity and duration of negative MP balance is based upon milk and milk protein production compared to feed intake in early lactation.

Postpartum dairy cows that were abomasally infused with AA with a similar profile to casein had increased milk production and milk protein yields compared with cows infused with water (Larsen et al., 2015). The uptake of essential AA by the mammary gland increased when cows were infused with AA, indicating that supply of AA is the driving factor for uptake by the mammary gland. Although the supplemented cows were supplied with more essential AA, the output of these AA was also increased; therefore, the protein deficiency in early lactation was maintained. The increase in MP as a result of the AA infusions positively impacted production parameters without any effect on MP balance. While this study matched the AA profile of milk protein and had positive effects on milk protein production, intake of this amount of MP (700 g at 5 DIM and 500 g at 15 DIM) may be infeasible in early lactation when dry matter intake is limited. Therefore, several researchers have considered the effects of supplementing individual rumen-protected AA on production and health parameters.

■ Supplemental Amino Acids Fed to Dairy Cows During the Transition Period

While there are clear increases in the MP requirements of close-up dry cows and even more in early lactation compared with the far-off dry period, a considerable amount of research has shown the benefits of supplementing specific AA during the transition period. Methionine is generally considered the first-limiting AA for dairy cows (NRC, 2001) and is used for milk protein synthesis, lipoprotein synthesis in the liver, and antioxidant synthesis. Lipoprotein synthesis is especially important in the transition dairy cow because lipoproteins are required for fat transport and may be critical as cows are accumulating fat in their liver as a result of adipose tissue mobilization due to negative energy balance. Supplemental methionine during the dry period had positive effects on milk yield and milk protein concentration with a corresponding increase in dry matter intake in early lactation (Osorio et al., 2013; Zhou et al., 2016; Batistel et al., 2017).

Supplemental methionine during early lactation increased milk protein concentration (e.g. Casper and Schingoethe, 1988) and a meta-analysis conducted by Zanton et al. (2014) demonstrated that protected methionine fed to lactating cows, including early lactation, consistently increased milk protein yield and concentration compared with control diets. However, others have reported supplementation of rumen-protected methionine had no effect on milk protein or any other production parameters (e.g., Chen et al., 2011; Lee et al., 2019).

Supplemental rumen-protected lysine in the prepartum period resulted in increased milk and component yields (Fehlberg et al., 2020). In this study, feeding lysine prepartum had a greater effect on early lactation than supplementing postpartum, with minimal effects when animals were only supplemented with additional lysine postpartum. Supplementing rumen-protected lysine to close-up and early lactation cows increased dry matter intake both pre- and postpartum. Postpartum, free fatty acid (FA) and BHB concentrations were reduced when rumen-protected lysine was supplemented with no differences in milk production parameters (Girma et al., 2019). The reduced free FA and BHB concentrations may be a result of carnitine synthesis, the methylated form of lysine, because carnitine aids in oxidation of FA in the liver and may therefore reduce fatty liver and ketone body formation (Carlson et al., 2006). These differences in ketone bodies postpartum were more evident with a high energy prepartum diet, indicating that supplementation of rumen-protected lysine pre and postpartum may have variable effects depending on other nutrients in the diet.

Mammary uptake of branched-chain AA (BCAA; leucine, isoleucine, and valine) and arginine from circulation greater than needed for milk protein synthesis in the lactating cow indicates increased catabolism of these AA in the mammary gland to produce glutamine, alanine, aspartate, asparagine and proline (Rezaei et al., 2016). However, the limited research on supplementing rumen-protected BCAA in the postpartum period has demonstrated moderate effects on reducing hyperketonemia events with insignificant changes in milk or milk protein yield (Leal Yepes et al., 2019). Additionally, muscle protein is lower in several AA, including BCAA, relative to milk protein, so skeletal muscle, although an important contributor, is not an ideal match for the milk AA profile (Table 1). We must rely on dietary and microbial AA sources to meet the AA deficits from mobilized skeletal muscle.

Table 1. Ratio of essential AA in skeletal muscle and milk protein in dairy cattle.

Amino Acid	Ratio of muscle AA ¹ / milk AA ²
Arginine	2.15
Histidine	0.99
Isoleucine	0.54
Leucine	0.81
Lysine	0.92
Methionine	0.81
Phenylalanine	0.82
Threonine	1.13
Tryptophan	0.53
Valine	0.75

¹Muscle AA reported by Doepel et al., (2004).

²Milk AA reported by Waghorn and Baldwin, (1984)

The variable responses from supplementing rumen-protected AA indicate there are interactions between the supply of AA and other nutrients. Additionally, some of the variation may be caused by our inability to accurately model MP in the transition dairy cow and the discrepancies between models for calculating MP. Cows have considerable variation in their metabolic efficiencies that likely contribute to the inconsistency in response with any supplemented nutrient, including AA. However, supplementing MP above the recommendation from NRC (2001) or some rumen-protected AA have shown the potential to improve both health and production outcomes in the transition dairy cow.

■ How Much Protein do Cows Lose During the Transition Period?

Dairy cows lose an estimated -3 to 17% of their BW from one to five weeks postpartum (Zachut and Moallem, 2017). This would indicate that a 700 kg dairy cow may lose > 100 kg through peak milk production. Some cows through early lactation actually increase their BW. There is considerable variation in BW change in early lactation, so a genetic component that is unaccounted for on commercial dairy farms likely exists (Friggens et al., 2007). Cows that gained weight had improved conception rate; however, they had reduced milk yield through the first 30 DIM (Zachut and Moallem, 2017). Variation in BW loss exists for animals consuming the same diet because of differences in intake and milk yield resulting in changes in energy balance. However, in individual cows, there are marked differences in efficiency of metabolic pathways that allow two cows consuming the same dry matter and producing the same milk output to have differences in BW change.

The main tissues mobilized are adipose tissue and skeletal muscle (protein). Adipose tissue is mobilized to meet energy requirements, whereas protein can be used to meet AA requirements and to meet energy requirements because AA can be converted to glucose and/or ketone bodies depending on the specific AA. A study that fed a protein-restricted diet to achieve a nitrogen balance of zero estimated that the labile supply of protein that can be mobilized is approximately 27% of empty body protein (Botts et al., 1979). However, studies that fed diets that supplied more protein estimated lower amounts of protein mobilized from calving through early lactation, with only 20% of empty body protein mobilized (Komaragiri and Erdman, 1997; van der Drift et al., 2012). Much like the variability in BW loss, the extent of protein mobilized is highly variable in transition dairy cows, with some cows losing considerably more than 20% and others gaining muscle depth during this period.

■ Measuring Protein Mobilization

The amount of protein stored in skeletal muscle has been documented best in studies that have evaluated carcass characteristics of dairy cattle at different stages of lactation. Commercial dairies and most researchers will not have access to these data on individual dairy farms; therefore, alternatives to assess the amount of empty body protein at specific time points is a way to estimate body reserves and therefore tissue mobilization. In beef cattle, ultrasound measurements of the longissimus dorsi muscle depth have been highly correlated to whole body protein (Greiner et al., 2003). Ultrasounds of dairy cattle at this location as well as other locations have been used to assess the amount of muscle depth with extrapolation to whole body protein (Schroder and Staufenbiel, 2006; van der Drift et al., 2012).

Metabolites related to AA metabolism can be measured in transition dairy cows to estimate the amount of muscle mass and proteolysis that is occurring. Muscle mass can be estimated from creatinine, the waste product produced by muscle from the breakdown of creatine and phosphocreatine (Wyss and Kaddurah-Daouk, 2000). This step is nonreversible and nonenzymatic, with relative increases in concentration indicating muscle accretion and relative decreases in concentration indicating muscle mobilization between two time points. During muscle degradation, 3-methylhistidine (3-MH) is produced and can be quantified to indicate muscle mobilization. Because muscle is constantly being degraded and accreted there will always be 3-MH present; however, relative differences between time points indicates more or less protein mobilization. To standardize across animals of different sizes, 3-MH relative to creatinine concentrations would indicate relative differences in protein mobilization per unit of muscle mass; the higher the ratio the more protein being mobilized. Feeding additional MP for the three weeks postpartum resulted in reduced 3-MH, indicating less muscle degradation in the period of time when MP balance is most negative (Carder and Weiss, 2017).

Measuring BW and body condition score through the transition period is likely to be used on commercial farms to measure muscle mobilization. While this is not specific to measuring whole body protein, animals in negative energy balance mobilize both adipose and muscle because of insufficient nutrient intake. Some commercial farms already implement reproductive protocols based on BW loss; animals that lose the most BW will have an increased voluntary waiting period to increase conception rates by delaying first

insemination to a time when they are more likely to conceive. As technology increases on farms and the ability to routinely monitor BW and body condition scoring increases, the ability to assign cattle to reproductive protocols depending on inputs like milk production and change in BW and body condition scoring will also increase.

■ Muscle Depth Loss from Transition Dairy Cows

Protein mobilization starts before parturition as intake of dietary protein is reduced and fetal and colostrum requirements for AA are increased (van der Drift et al., 2012). Considerable variation in the amount of longissimus dorsi muscle depth has been recorded in transition dairy cattle, with a minimum of approximately 2 cm and a maximum of 6.5 cm (McCabe, 2020). While we generally do not think of muscle as being a storage tissue for AA as adipose is for FA, the variation between cows indicates that some animals store more AA in muscle than others. Dairy cows appear to mobilize muscle between -7 and 30 DIM (Figure 2), with less variation at 30 and 60 DIM compared with prepartum time points and maintain a minimum depth of approximately 2 cm at the longissimus dorsi location. A study was conducted with prepartum cows to evaluate the effects of exercise on muscle and liver composition and plasma concentrations of metabolites and hormones. Exercising dairy cattle during the prepartum period caused physiological adaptation with an increase in muscle and liver triglycerides and plasma NEFA and a reduction in plasma BHB and insulin concentration (Davidson, 2002). Using exercise as an environmental change for the cows, the authors noted differences in metabolic response between animals that were exercised and those that were not exercised and suggested that increasing muscle tone may have benefits for reducing metabolic diseases due to changes in metabolites and insulin concentration. Very little is known about if, and to what extent, we can alter muscle depth in cattle through nutrition and management; however, limited work with exercising cattle has demonstrated that this may positively impact the metabolic status of prepartum dairy cattle.

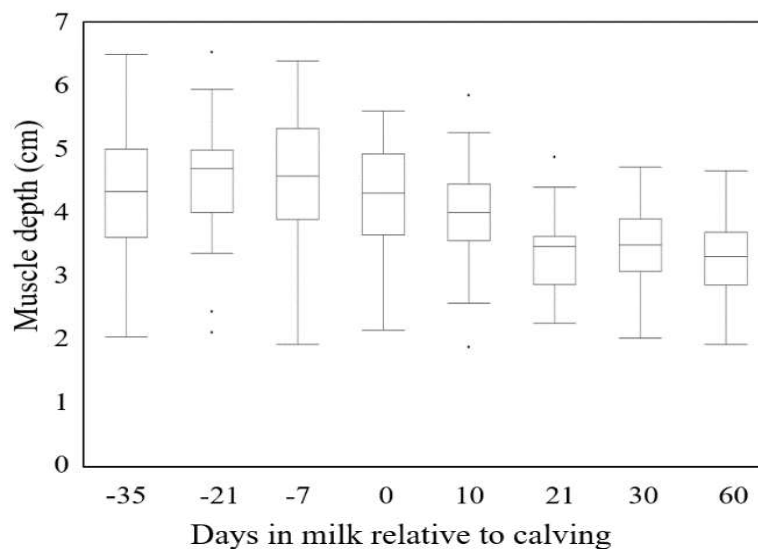


Figure 2. Box and whisker plot of muscle depth relative to calving for multiparous Holstein cattle (n=48). The horizontal line within the box represents the mean, the upper box represents the 75th percentile, the lower box represents the 25th percentile. The whiskers represent the minimum and maximum excluding outliers with outliers identified as individual points. Adapted from McCabe, 2020.

Cows with higher BCS i.e., more adipose tissue, are more likely to have reduced dry matter intake and increased likelihood of developing metabolic diseases such as ketosis and fatty liver. Less is known about the consequences of animals mobilizing excessive muscle during the transition period. In early lactation,

the use of alanine specifically and AA generally for gluconeogenesis is increased because of the increased demand for glucose (Overton et al., 1999). The use of AA for glucose synthesis in early lactation when MP is insufficient from intake suggests some AA are likely mobilized from skeletal muscle.

In a relatively small dataset, we observed that there was a moderate negative correlation between muscle depth of the longissimus dorsi location three weeks before calving and the amount of muscle depth that is lost from three weeks before calving to one month postpartum (adapted from McCabe, 2020; Figure 3). Cows with lower muscle depth prepartum gained muscle depth during this time while cows that had higher muscle depth tended to lose more muscle. Similar results have been reported by van der Drift et al. (2012) with animals with greater muscle depth prepartum having increased mobilization of muscle postpartum. Although we generally do not see skeletal muscle as an AA store, the variation in the extent that animals mobilize indicates that there are factors that regulate both catabolism and anabolism that can be influenced by environment, diet, and genetics.

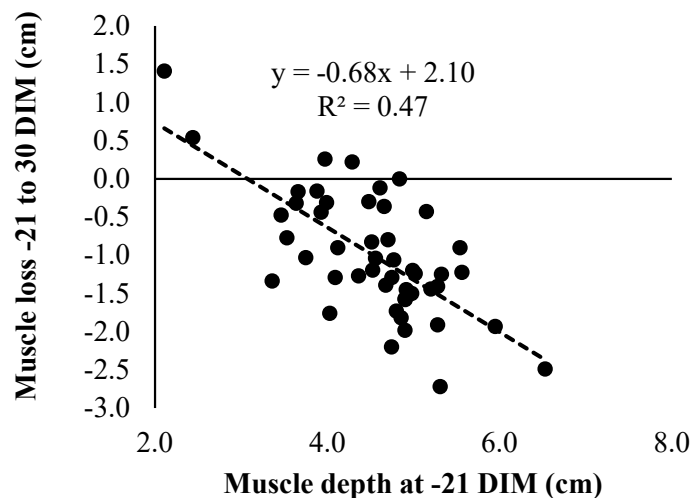


Figure 3. Muscle loss from -21 to 30 DIM vs. muscle depth at -21 days prior to expected calving for multiparous Holstein cattle (n=48). Adapted from McCabe (2020).

■ Energetics Around Losing and Gaining Muscle

The energy requirements for maintenance are based on metabolic BW multiplied by a constant coefficient of 0.080 Mcal/kg BW^{0.75}. As an animal increases or decreases in muscle mass, the amount of energy required for maintenance is impacted. The proportion of empty body fat increases as BCS increases whereas the relative proportion of empty body protein is thought to be reduced as BCS increases (NRC, 2001). Energetic inefficiency exists when an animal is converting AA to muscle and mobilizing muscle into AA. Therefore, although animals are able to use skeletal muscle to meet MP requirements in early lactation, this inefficiency needs to be accounted. The MP requirements for increasing muscle synthesis when MP balance is positive also needs to be taken into account. Liu and VandeHaar (2020) suggested that considerable variation exists for a cow's ability to capture protein in both milk and body tissue. They proposed there is likely a genetic component to the ability of cattle to capture dietary protein and convert it into body protein. When formulating diets for transition dairy cattle, the change in BW, and therefore changes in adipose and muscle tissue amounts, need to be accounted for regarding the nutrients supplied by mobilization of tissue (energy and AA in the case of muscle) and the energy and MP required to regain this tissue at other stages of lactation.

▪ Relationship Between Muscle Loss and Oxidative Stress

The transition period is marked by a considerable amount of disease because the cow is unable to adapt to the new physiological normal. This period of time is characterized by increases in oxidative stress, further exacerbating the risk for metabolic diseases. As previously mentioned, supplementation of rumen-protected methionine benefits production, and reduces oxidative stress and immunological markers in the transition period (Osorio et al., 2014). As tissue becomes more metabolically active, in late gestation and early lactation, more oxygen is consumed and the mobilization of adipose tissue leads to an increase in reactive oxygen species (Abuelo et al., 2019). Oxidative stress contributes to inflammation, which may increase the requirements of AA to be mobilized from muscle. However, we know less about the ability of protein mobilization to directly contribute to oxidative stress compared with the ability of adipose tissue mobilization. Supplementing rumen-protected methionine has been linked to both reducing oxidative stress and reducing protein degradation. Work done in humans with sarcopenia or muscle wasting (which I admit is not a perfect match for protein mobilization in dairy cows in early lactation but does represent the magnitude of muscle loss that dairy cattle experience) has demonstrated links between oxidative stress, inflammation and muscle atrophy (Meng and Yu, 2010). This work in humans suggests at least that increased oxidative stress and inflammation in maladapted dairy cattle may contribute to more muscle mobilization in early lactation.

▪ Conclusions

Because of the increased demands for AA in late gestation and early lactation, there is a relative consensus for increasing MP above NRC (2001) requirements for close-up dry cows. Insufficient intake of MP in early lactation causes cows to mobilize skeletal muscle to meet the AA gap. Increasing the MP available in early lactation will preferentially allocate AA to the mammary gland, with some variation in response evident from supplementing individual rumen-protected AA. Dairy cattle can mobilize a considerable amount of muscle; however, it appears that there is a minimum amount of muscle depth they must maintain. While muscle mobilization may start before calving, there appears to be little difference between 30 and 60 days postpartum indicating cows likely are not using skeletal muscle to a large extent after 30 DIM. Skeletal muscle can be stored and mobilized to meet MP requirements in early lactation. To truly model requirements in a dairy cow, the extent of tissue that is mobilized needs to be accounted for during mobilization and re-accretion as well as the energetic inefficiencies around protein catabolism and anabolism.

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Effects of Medium Chain Fatty Acid Supplementation on Dairy Cow Performance and Rumen Fermentation

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Medium chain fatty acids (MCFA) are fatty acids with chain lengths between 6-12 carbons. Human research suggests that MCFA may affect energy partitioning and prevent obesity, but effects of MCFA supplementation in diets of lactating dairy cows have not been extensively studied. The objective of this study was to evaluate the effects of MCFA supplementation on performance, digestibility, and rumen fermentation of lactating dairy cows. Thirty ($n = 8$ primiparous, $n = 22$ multiparous) Holstein dairy cattle in mid lactation (637 ± 68.5 kg of body weight, 98.5 ± 27.4 days in milk; mean \pm SD) were used in a crossover design with 25-d diet adaptation and 3-d data collection periods. Cows were supplemented with MCFA at 0.25% of dietary dry matter (TRT) or additional dry ground corn replacing MCFA (CON). No differences were observed in dry matter intake, total tract nutrient digestibility or body weight between TRT and CON. However, there was a tendency towards a negative correlation between pre-trial milk yield and animal response to TRT in BW change ($P = 0.06$), where high producing cows increased BW to a lesser extent when fed MCFA. Milk yield did not differ between treatment groups but primiparous cows decreased lactose yield in TRT compared to CON (1.45 v. 1.51 kg/d; $P < 0.01$). Fat and protein yield did not differ between treatments. However, a negative correlation was found between pre-trial milk yield and animal responses to TRT in protein yield ($P = 0.04$), in which TRT decreased milk protein yield in higher-producing cows, but not in lower-producing cows. Minimum rumen pH tended to be higher in TRT compared to CON (5.66 v. 5.54 ; $P = 0.08$), whereas duration of acidosis (<5.8 , min/d) did not differ between treatment groups. Total rumen volatile fatty acid concentration and its profile did not differ between treatment groups. These findings suggest that inclusion of MCFA in diets of lactating dairy cows may not increase production performance but may decrease risk of rumen acidosis.

Heifers with Short Anogenital Distance have Better Fertility than those with Long Anogenital Distance

J.E. Carrelli*, M. Gobikrushanth†, M. Corpron‡, I. Rajesh*, W. Sandberg‡, M.G. Colazo§*, A. Ahmadzadeh‡, M. Oba*, and D.J. Ambrose§*¹

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In today's environment, when replacement heifers are in oversupply, a reduction in age at 1st calving could improve profitability by reducing rearing costs up to \$3.25/hd/d. Anogenital distance (AGD, the distance from the anus to the clitoris in cattle) is a phenotype determined by exposure to male sex hormones during fetal development. Earlier studies with limited numbers of cows and heifers have shown that those with long AGD are less fertile than their short AGD counterparts. This expanded study was to determine if the same negative relationship exists between AGD and fertility measures in a larger group of maiden Holstein heifers. AGD was measured in 1,692 heifers where mean (\pm SD) age at measurement was 13.9 ± 1.5 mo. AGD was normally distributed with a mean of 107.3 ± 10.5 mm (range, 69 to 142 mm). Heifers were categorized into short (≤ 110 mm) and long (> 110 mm) AGD groups based on the optimum threshold AGD predictive of pregnancy to 1st AI (P/1stAI), and associations with fertility were determined. Heifers with short AGD required fewer services per conception (1.5 ± 0.1 vs. 1.7 ± 0.1 ; $P < 0.01$), conceived earlier (14.9 ± 0.2 vs. 15.1 ± 0.2 mo; $P < 0.01$), and had greater P/1stAI (58.3 ± 3.0 vs. 49.6 ± 3.1 %; $P < 0.001$) than those with long AGD. Moreover, heifers with long AGD had reduced pregnancy risk up to 15 mo than those with short AGD (hazard ratio: 0.59; $P < 0.001$). In summary, heifers with short AGD were more fertile than those with long AGD when services per conception, age at conception, P/1stAI, and cumulative pregnancy risk up to 15 mo of age were considered. These findings strengthen the potential for AGD to be used as a fertility trait and management tool in future selection programs.

Take Home Messages: (1) Heifers with short AGD have improved fertility compared with heifers with long AGD (2) Heifers with short AGD may offer an economic advantage through reduced rearing costs.

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Managing High-Straw Dry Cow Diets to Optimize Health and Performance

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■ Take Home Messages

- ▶ Controlled-energy, high-straw dry cow diets may be successfully used to minimize body condition change in the dry period and improve metabolic health post-calving.
- ▶ Rumen health and energy balance can be improved post-calving by promoting greater and more consistent intake of high-straw diets in the dry period.
- ▶ Increasing the physical density of high-straw dry cow diets, by minimizing straw chop length and adding water, will reduce sorting and promote greater intakes.
- ▶ Addition of molasses-based liquid feed to high-straw dry cow diets will improve palatability, reduce sorting, and promote greater intakes.
- ▶ Proper feed bunk management to ensure good and continuous feed availability and access is essential for maintaining the success of these high-straw dry cow diets.

■ Introduction

The transition period, typically three weeks pre- and post-calving, is a vulnerable time for the dairy cow because she experiences several physiological, metabolic, and behavioural challenges (Drackley, 1999). At the onset of calving, the cow's nutrient demand drastically increases in an attempt to meet the demands of milk production. At the same time, and in the weeks leading up to calving, cows will reduce their dry matter intake (DMI) due to both behavioural and physiological stressors. Combined, the decrease in DMI prior to calving, the increase in nutrient demand, and the slow rate in which DMI increases in the weeks following calving, put cows into a state of negative energy balance (NEB). This state of NEB is inevitable, and all cows will experience it to some degree simply because energy intake does not match energy output in the weeks following calving. However, the degree to which cows will experience NEB will influence her risk of experiencing metabolic disease (i.e., ketosis) and will ultimately dictate her success through the transition period. Ketosis is a disease experienced in early lactation that affects 26-55% of cows at the subclinical level (McArt et al., 2015), and can result in lower milk production (McArt et al., 2012), reduced reproductive performance and increased risk of other illnesses. The risk of excessive NEB and ketosis in early lactation has been associated with reduced feeding activity, rumination, and DMI in the weeks leading up to calving (Goldhawk et al., 2009; Kaufman et al., 2016a). Similarly, changes in pre-partum feeding behaviour have been associated with greater risk of metritis and other health disorders post-calving (Huzzey et al., 2007; Luchterhand et al., 2016). These findings highlight the importance of maximizing intake in the late dry period, while also promoting desirable feeding behaviours.

One of the risk factors associated with metabolic and infectious disease post-calving, and also associated with reduced DMI, is pre-calving body condition score (BCS) (Invgartsen, 2006; McArt et al., 2012; Roche et al., 2015). Duffield (2000) concluded that cows who were over-conditioned pre-partum were at 1.6x greater risk of developing subclinical ketosis post-partum. This association is thought to be a result of over-conditioned cows tending to eat less because of elevated plasma leptin concentrations. Over-conditioned cows are also more sensitive to lipolytic stimuli, and given that they naturally have more body fat to mobilize,

they are at greater risk of excessive body fat mobilization. Increased levels of body fat mobilization can quickly overwhelm the liver and, through a cascade of metabolic processes, can ultimately result in ketosis, decreased DMI, and further metabolic issues. On the other hand, losing BCS during the dry period also has its risks, including poorer reproductive performance post-calving, and increased chances of needing antibiotic treatment (Chebel et al., 2018). Evidently, managing BCS in the dry period such that cows neither lose nor gain, but rather maintain body condition, is a true balancing act that requires careful consideration. Over the past 20 years, extensive research has been focused on feeding dry cows to 100% of their nutrient (primarily energy) requirements (Dann et al., 2006; Janovick et al., 2011). This concept has become very popular in the industry and has resulted in improved energy balance post-calving. This research has provided strong evidence that maximizing DMI, while minimizing body condition loss or gain, is a key area in nutritional management of the transition cow.

■ High-Straw Dry Cow Diets

One widely accepted strategy to control energy intake in the dry period is to incorporate large amounts of low-nutrient dense feedstuffs, such as wheat, oat, or barley straw, into the diet (Beever, 2006; Dann et al., 2006; Janovick et al., 2011). This type of diet is referred to as a 'controlled energy dry cow diet', or is also commonly referred to as the 'Goldilocks diet'. Similar to the story of Goldilocks and the three bears, these diets are based on the concept of not too much, and not too little, but just the right amount – of energy, that is. The goal of these diets is to control energy consumption and maintain BCS, and thus, promote metabolic health, while also encouraging voluntary intake, feelings of satiety, and rumen fill. Dann et al. (2006) demonstrated that by using this type of controlled energy dry cow diet (cows fed to 100% of their requirements), cows had less fat mobilization and, as result, lower blood beta-hydroxybutyrate (BHB) concentrations in the first ten days post-partum compared with cows that were fed to 150% of their energy requirements. Likewise, Janovick et al. (2011) reported cows that were overfed energy in the dry period had greater blood BHB levels and experienced greater lipid mobilization post-calving. Continued research has supported this concept (Richards et al., 2020); thus, undoubtedly, these diets work from a metabolic standpoint.

However, from a feeding behaviour standpoint, there are areas of concern. For example, straw is considered unpalatable to the cow, which could increase the risk of sorting. Sorting in the dry period not only increases the risk that cows do not end up consuming the intended diet (and thus the targeted nutrient consumption), but if carried over into lactation, could be cause for concern because the risks for poor metabolic health (especially ruminal acidosis) are greater then. Very little research has investigated how pre-calving feeding behaviour influences post-calving feeding behaviour; however, past research with young calves has shown that if calves learn a behaviour prior to a dietary change they are more likely to carry that behaviour over following the transition onto a new diet. Moreover, straw is bulky and when incorporated at high rates, particularly with long particle size, can extend rumen retention time and increase rumen fill (Drackley, 2007). From a physiological standpoint, this could be beneficial given that the slower passage rate could improve rumen mat formation, but from a behavioural standpoint, the longer the forage remains in the rumen, the less feed the cow can consume (Drackley, 2007). And lastly, large amounts of dry straw make the dry cow diet very different from the fresh cow diet in terms of physical characteristics. The pre-fresh diet is often bulky and lower in moisture content, whereas the fresh cow diet is typically denser with greater moisture content. These differences, coupled with significant drops in DMI in the weeks leading up to calving, can make it particularly difficult for cows to adapt to this dietary change, especially from a behaviour standpoint. Despite this being a challenge of controlled-energy dry cow diets, there are opportunities to make the pre- and post-fresh diets more similar to one another by manipulating certain physical characteristics. Because cows have well-defined feeding patterns, minimizing the behavioural stress associated with the transition from the pre-fresh to the post-fresh diet could also reduce the physiological and metabolic stress cows experience during this period.

■ Managing Physical Characteristics of High-Straw Dry Cow Diets

In a series of three studies on feeding high-straw dry cow diets we observed that by manipulating forage particle size, moisture content, and overall palatability we can minimize the physical differences between the pre- and post-fresh diets, while making these dry diets more desirable to the cow (Havekes et al., 2020a, Havekes et al., 2020b, Havekes et al., 2020c).

In the first study, we fed dry cows high-straw dry cow diets that were nutritionally the same but differed in the chop length of the wheat straw (Havekes et al., 2020a). Half the cows received a diet with straw that was chopped with a 2.54 cm (1-inch) screen, and the other half received a diet with straw that was chopped with a 10.16 cm (4-inch) screen. Cows were fed their respective diet for the entire dry period, and then fed the same lactating diet for 28 days post-calving. Cows fed the diet with the shorter chopped straw had greater DMI across the dry period. Little research has looked at differing forage particle size in the dry period, but our result is consistent with work on shorter corn silage particle size in lactating diets (Kononoff et al., 2003). Of particular interest in our work was the noticeable difference in intake as cows approached calving (Figure 1). Cows fed the shorter chopped straw were able to maintain more consistent intake in the seven days leading up to calving compared with cows fed the longer chopped straw.

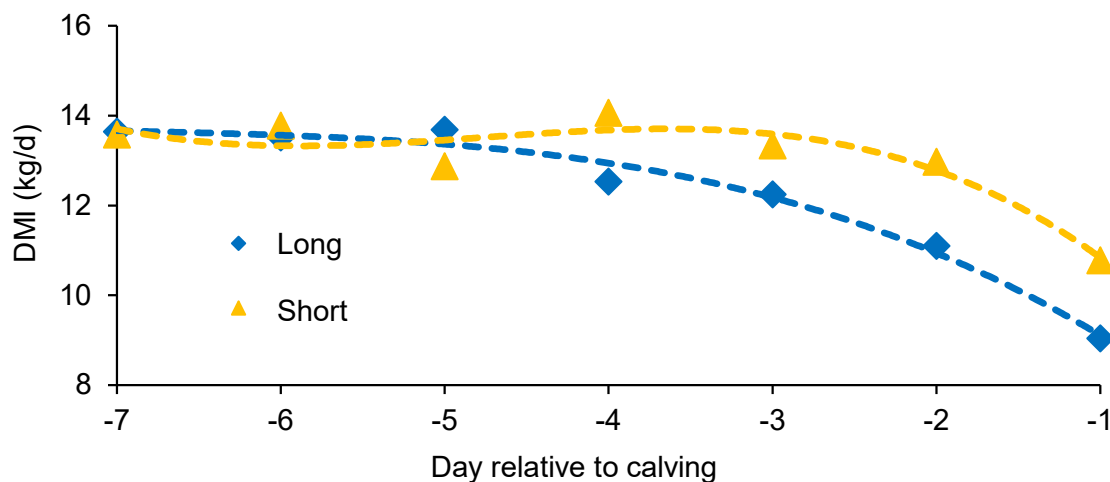


Figure 1. Daily DMI in the week leading up to calving when cows were fed dry cow diets differing in the chop length of wheat straw: 1) chopped with a 2.54-cm screen (Short), or 2) chopped with a 10.16-cm screen (Long). Adapted from Havekes et al. (2020a).

Regardless of chop length, cows sorted against the longest forage particles in the dry diets, but unsurprisingly, cows fed the short-chopped straw sorted less. This result is consistent with past research investigating the impact of straw particle size on sorting behaviour (Coon et al., 2018; Dancy et al., 2019). Interestingly, cows fed the short-chopped straw actually sorted against the fine fraction of the diet during the dry period. This is surprising because the fine fraction typically contains the small grain components that cows deem the most palatable; as a result, we generally see cows sorting in favour of this fraction (Miller-Cushon and DeVries, 2017). This finding may be partially explained by the fact that the short-chopped straw contributed a large amount of straw dust to the fine fraction, presumably making it less appealing to the cow. Similar results were recorded by Montoro et al. (2013), where calves were fed either fine or coarse chopped hay, and calves fed the fine chopped hay sorted more against the fine fraction, and by Coon et al. (2018) when lactating cows sorted against the fine fraction when fed shorter chopped straw. It is noteworthy that rumen health was improved in our study during the first week of lactation for cows fed the short-chopped straw dry cow diet, as evidenced by less of a decline in mean rumen pH level following the transition onto the lactating diet. This may have partially been driven by the consistency in intake for

those cows in the week leading up to calving. Moreover, cows fed the short-chopped diet had larger meals throughout the dry period; this may have increased the absorptive capacity of the rumen and primed the rumen for larger meals post-calving. Lastly, the dry cow diet with short-chopped wheat straw resulted in better metabolic health post-calving, as demonstrated by a tendency for cows to have lower blood BHB levels three weeks post-calving. By maintaining a more consistent intake in the week before calving, cows fed the short-chopped straw dry cow diet exhibited improved energy balance post-calving.

Despite the shorter straw particle size in the dry cow diet improving intake, particularly in the week leading up to calving, and minimizing sorting, a significant amount of sorting was still observed in that study (Havekes et al., 2020a). Another strategy thought to minimize sorting activity is water addition because the water may help bind the smaller particles to the larger particles making the diet physically more difficult to sort (Leonardi et al., 2005; Felton and DeVries, 2010; Fish and DeVries, 2012). Reduced sorting was observed by Leonardi et al. (2005) when water was added to a lactating cow diet containing only dry forages. Because controlled energy dry cow diets contain a large proportion of dry forages, they may also benefit from additional water. Another potential benefit is that water addition may help to further minimize the physical differences between the dry and lactating diet by making the DM%, and thus overall density, more similar between the two diets.

In our second dry cow study we fed cows either a high-straw dry cow diet with no water added (DM = 53.5%) or a high-straw dry cow diet with water added to decrease the DM content by approximately 10% (DM = 45.4%). Based on the positive results from our first study, we used straw that was chopped with a 2.54-cm (1 inch) screen. Cows fed the diet with added water had greater intake across the dry period and were able to maintain more consistent intake in the week leading up to calving compared with cows fed the control diet. The hypothesis in our work, as well as the work done with lactating diets, is that water may help bind the smaller ration particles to the larger particles making the diet physically more difficult to sort. In our study, the particle distribution was the same for all four fractions between the two treatment diets, suggesting that water did not have an adhesive effect on the smaller particles. Despite this, water addition did minimize the degree to which cows sorted against the longest forage particles. To our knowledge, no researchers had previously investigated the effects of adding water to a high-straw dry cow diet. When water was added to a lactating ration, Felton and DeVries (2010) reported decreased intake and increased sorting, whereas Leonardi et al. (2005) reported decreased sorting. The inconsistency in results is likely related to the composition and originating DM of the diets, and we may conclude that water addition is more beneficial when working with diets higher in DM and dry forages.

Lastly, cows fed the diet with added water had greater mean rumen pH during the first week post-calving (Figure 2) and tended to have greater mean rumen pH during the second and third week after calving. This finding is also similar to the first study and, once again, may be attributed to more consistent intake (amount and composition because of reduced sorting) in the week leading up to calving, as well as greater intake across the entire dry period. Greater intakes can help develop the rumen papillae and increase the absorptive capacity of the rumen, which in turn can prevent the rumen pH from dropping too low following the transition onto the lactating diet (Kleen et al., 2003; Derakhshani et al., 2017).

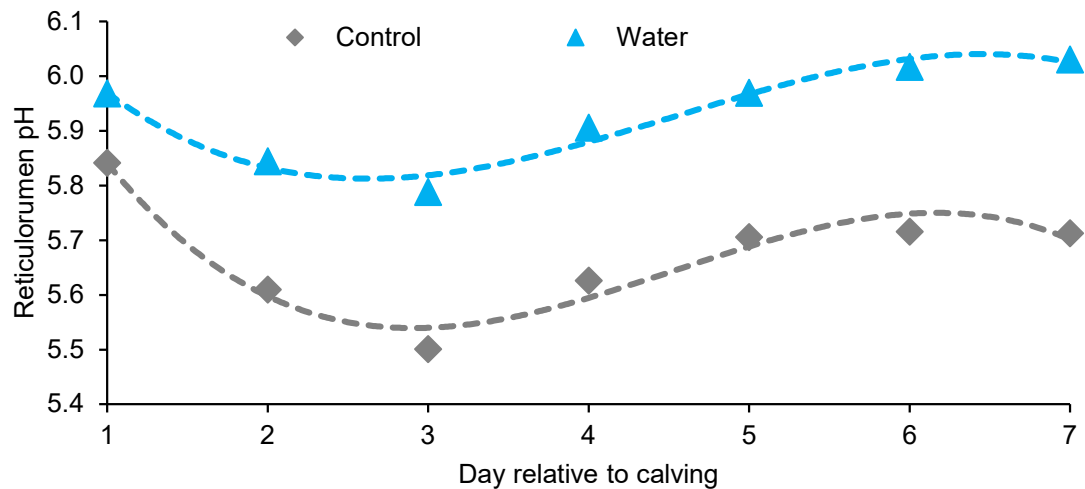


Figure 2. Mean daily reticulorumen pH for cows one-week post-calving when fed dry cow diets that differed in DM content (Control: TMR with 54.5 % DM; Water: TMR with 45.4 % DM). Adapted from Havekes et al. (2020b).

After investigating how manipulating physical characteristics of the dry cow diet (changing density through particle size and water addition) impacted feeding behaviour and performance, we wanted to determine if improving the overall palatability of the diet may have even more beneficial impacts on transition cow performance. Feeding sugar, often in the form of molasses, has been shown to have several benefits for lactating dairy cows. These benefits include improved microbial efficiency, higher intake, and reduced sorting (Oba, 2011; DeVries and Gill, 2012). Molasses also supports fibre-digesting bacteria; given that controlled-energy dry cow diets are high in lesser-digestible fiber, molasses supplementation in these diets could be particularly advantageous. Additionally, dairy cows prefer sweet tasting flavours and may be more likely to have higher intake when fed a diet with added molasses (Chiy and Phillips, 1999). Combined, these benefits could greatly increase the cow's success throughout the transition period, but to our knowledge, very little research had been done to determine the impact of molasses supplementation in dry cow diets.

For our third study, we fed cows a high-straw dry cow diet with either: 1) no liquid feed supplementation, or 2) molasses-based liquid feed supplementation at 1.0 kg/cow/day (DM basis) (Havekes et al., 2020c). When fed the diet with added molasses, cows had greater intake across the dry period and maintained more consistent (and greater!) intake in the week leading up to calving compared with cows fed the control diet (Figure 3). This is consistent with some other research done with molasses supplementation to dry cows (Miller, 2011), but inconsistent with others (Litherland et al., 2013). The differences observed between the research groups are likely related to composition of the diets (including dietary cation-anion difference and starch levels). However, greater intake by cows when fed molasses, especially in high NDF and low fermentable diets, makes sense given the fibre-digesting benefits of sugar.

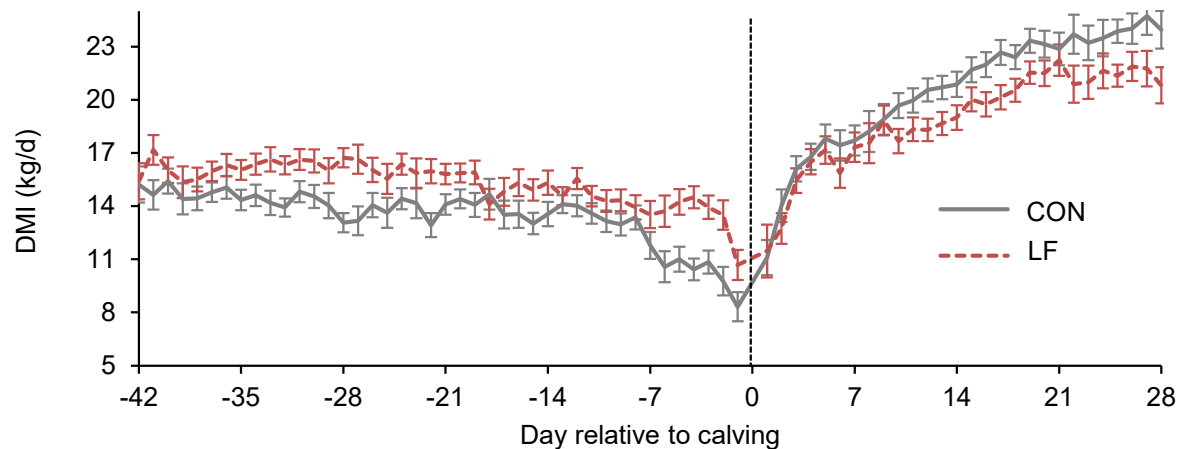


Figure 3. Mean (\pm SE) daily DMI (kg/d) for cows fed 1 of 2 dietary treatments during the dry period: (CON: dry diet with no molasses supplementation, LF: dry diet with molasses supplementation at a rate of ~ 2.0 kg/cow/d as fed (1.0 kg/cow/d DM)); upon calving all cows were fed the same lactating cow ration. Vertical line represents calving. Adapted from Havekes et al. (2020c).

Cows fed the molasses also had a faster feeding rate in the dry period. Typically, faster feeding rates are not desirable as they are associated with slug feeding and sub-acute ruminal acidosis (Grant and Albright, 2000; DeVries, 2019). However, these negative consequences are more likely to occur when cows are fed a rapidly fermentable diet, such as the lactating diet, rather than a high-forage dry cow diet. Interestingly, after calving, cows did not carry over this feeding behaviour. The observed increased feeding rate on the dry diet was likely influenced by the fact that cows prefer sweeter tasting flavors and are more likely to consume those feeds faster (Chiy and Phillips, 1999), and spend less time sorting. Regardless of treatment, in the dry period, cows sorted against the long forage particles, but cows fed the diet with added molasses sorted against this fraction less. This finding agrees with DeVries and Gill (2012) where molasses supplementation decreased sorting in lactating diets. Interestingly, in our dry cow study, this sorting behaviour carried through into lactation where we observed that cows previously fed the molasses actually did not sort for or against the long forage particles, but cows fed the control diet continued to sort against this fraction.

During the dry period, cows fed the molasses had higher rumen pH, which is rather surprising given the characteristics of the diets being fed. However, molasses has been shown to improve microbial efficiency and promote fibre-digesting bacteria, which may have promoted a healthier rumen environment (Penner and Oba, 2009; Oba, 2011). Post-calving, cows previously fed the diet containing molasses continued to maintain a more stable rumen environment, as demonstrated by higher mean rumen pH during the first two weeks of lactation. Molasses-fed cows had, on average, lower maximum blood BHB concentrations (0.9 mmol/L) in the first week post-calving. Cows on the control diet had an average maximum blood BHB concentration of 1.5 mmol/L. This maximum BHB level indicates that the control cows were, on average, experiencing subclinical ketosis (typically defined as blood BHB ≥ 1.2 mmol/L). Recent research suggests that at blood BHB levels ≥ 1.2 mmol/L cows are anywhere from 4.7 to 14.7 times more likely to develop clinical signs of ketosis (Benedet et al., 2019). This finding could be, in part, related to the lower intake of the control cows in the week leading up to calving; Goldhawk et al. (2009) reported that decreased intake in the week leading up to calving increases the cow's risk of developing ketosis in early lactation. Lastly, we observed a numerical increase of 2.5 kg/d 4% fat corrected milk yield (Figure 4), and 2.8 kg/d energy corrected milk yield, and an increase in efficiency of production (kg of milk per kg of DMI) by 0.2 percentage points for cows fed the diet with molasses. Similarly, Litherland et al. (2013) reported increased 3.5% fat corrected milk yield when cows were fed molasses throughout the dry period and for the first 56 days of lactation.

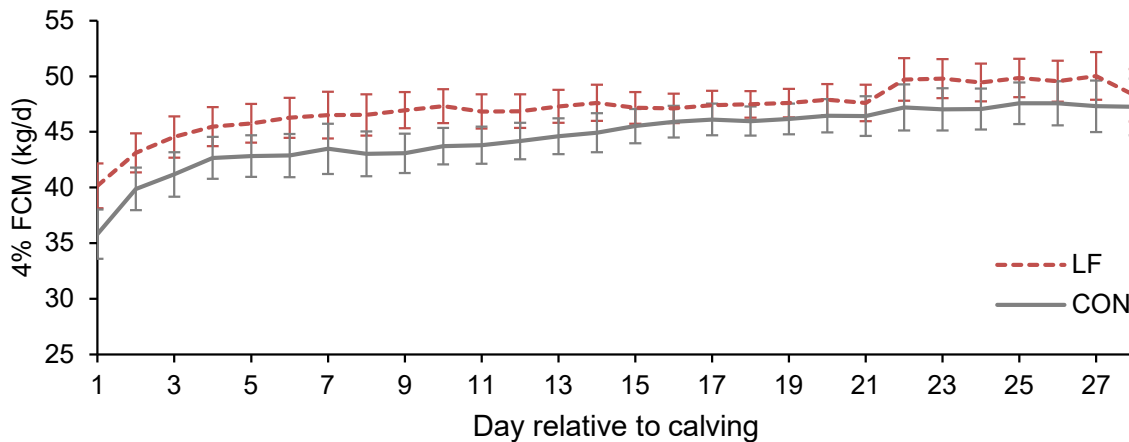


Figure 4. Mean daily (\pm SE) 4.0% fat-corrected milk yield (FCM; kg/d) for cows fed 1 of 2 dietary treatments during the dry period: (CON: dry diet with no molasses supplementation, LF: dry diet with molasses supplementation at a rate of \sim 2.0 kg/cow/d as fed (1.0 kg/cow/d DM)); upon calving all cows fed were the same lactating cow ration. Adapted from Havekes et al. (2020c).

■ Bunk Management of High-Straw Diets

We know that DMI is a function of feeding behaviour (DeVries, 2019), and to increase DMI we need to manipulate one or more aspects of feeding behaviour. Even with excellent nutritional management of controlled energy dry cow diets, outside factors such as bunk management can greatly influence the cow's feeding behaviour and, ultimately, her intake (DeVries, 2019). Much research has been focused on the impact of bunk management during the lactating period, but less research has been focused on this area in the dry period. Arguably the way the bunk is managed in the dry period may be even more critical than how it is during lactation for the cow's success through transition; therefore, it warrants consideration.

Proudfoot et al. (2009) investigated how increased competition at the feed bunk impacted intake and feeding behaviour of prepartum cows. Those researchers concluded that competition (as result of overcrowding the bunk) increased the frequency of displacements from the feed bunk and tended to reduce intake of multiparous cows in the week before calving (Proudfoot et al., 2009). When these close-up cows were fed in a competitive environment, they also had dramatically faster feeding rates compared with non-competitively fed cows (Hosseinkhani et al., 2008). Given the results of our dry cow studies (Havekes et al., 2020a,b,c) and those of Huzzey et al. (2007) and Goldhawk et al. (2009), it is evident that maximizing intake in the dry period, and particularly in the week leading up to calving, is a very important component to metabolic health post-calving. Thus, feed bunk competition should be minimized, ideally during the whole dry period, but especially in the weeks leading up to calving.

Another important component of bunk management that is well-researched for lactating cows is feed availability. Little research has been focused on feed availability in the dry period, but we can hypothesize that the feeding behaviour response would be similar to that in the lactating period. Feeding for no refusals, commonly referred to as 'slick bunk' feeding, is widely used (Silva-del-Río et al., 2010); however, this feeding strategy naturally results in cows having little or no access to feed for periods throughout the day. Feed restriction in the lactating period was studied by Collings et al. (2011), who concluded that slick bunk feeding coupled with competition doubled the amount of feed bunk displacements. Feed bunk competition also decreases daily lying time and increases non-feeding standing time (Huzzey et al., 2006; Proudfoot et al., 2009). These findings become increasingly important when we consider how lying activity in the pre-fresh period may influence post-calving performance. For example, Itle et al. (2015) reported that cows diagnosed with subclinical ketosis post-calving spent less time lying pre-calving. We also know that excessive standing time is a risk factor for lameness, which can be detrimental to the success of the

transition cow. In support of the potential link between stocking density, time budgets, and risk of excessive NEB, Kaufman et al. (2016b) demonstrated that increasing stocking density by 5% during the week pre-calving increased the risk of ketosis by 10%. Based on these results, and because maximizing DMI in the dry period is so critical, feeding dry cows for a slick bunk, especially under competitive conditions, is not recommended. In vulnerable times, such as the dry period and early lactation, it is recommended to provide ≥ 76 cm (30 inches) of feeding space per cow (DeVries, 2019) with continuous feed availability.

Feed push ups are also an important aspect of feed availability. Pushing up feed continuously throughout the day is critical for ensuring cows consume a balanced diet and that they have continuous access to feed (DeVries, 2019). Push ups can help promote consumption of a balanced diet by mixing up the feed in front of the cows and minimizing any existing locations of sorting. This is increasingly important when cows are fed a diet with ingredients that may be deemed less palatable, such as straw, and when diets are more easily sorted because of their physical characteristics.

Lastly, one final consideration for dry cow diets, especially if water is being added to the diet, is the potential for heating and spoilage. Decreased intake with water addition to lactating diets observed by Miller-Cushon and DeVries (2009) and Felton and DeVries (2010) was attributed to the TMR spoiling. Eastridge (2006) suggested that TMRs greater in moisture content may be less stable and, thus, more prone to spoilage, particularly during periods of warmer weather. To minimize these risks, careful attention must be placed on maintaining silage quality (in bunk and at feed out) and good feeding management. Dry cow diets should be mixed and delivered at least daily, and potentially more often in periods of high environmental temperature and humidity.

■ Conclusions

Controlled-energy dry cow diets are beneficial from a metabolic standpoint, as demonstrated by supporting research and adoption of this feeding strategy in the industry. Equally important, however, is the role that feeding behaviour plays in the cow's success through the transition period. High-straw dry cow diets are significantly different from the lactating diet in terms of physical characteristics and may be more prone to sorting. Manipulating the physical characteristics (i.e., greater density through smaller straw particle size and water addition) can make these dry diets more physically similar to lactating diets; this strategy has proved to be beneficial from both a behavioural and metabolic health standpoint. Further, improving the palatability of these high-straw diets through the use of a molasses-based liquid feed also proved to be beneficial for optimizing transition cow performance. Finally, nutritional considerations for these diets, albeit important, are only one piece of the puzzle. How these diets are managed at the feed bunk to ensure good and continuous availability and access also play a critical role in the overall success of the transition period.

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Effects of Dietary Butyrate Supplementation and Oral Non-Steroidal Anti-Inflammatory Drug Administration to Transition Cows on Performance, Plasma Metabolites and Reproduction

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Dairy cows experience negative energy balance in the transition period due to inflammation and high energy demands for milk production. This study evaluated the effects of dietary butyrate supplementation and oral non-steroidal anti-inflammatory drug administration on performance, plasma metabolites and reproduction in transition cows. Eighty-three cows were fed an iso-energetic diet containing calcium butyrate (1.42% of diet dry matter (DM)) or a control (1.04% palm fat and 0.38% calcium carbonate of diet DM) during the calving transition period from -28 to 24 DIM (calving = d 0). The closeup (CUD) diet contained 13.5% starch and 43.0% neutral detergent fiber (NDF), and the fresh diet contained 22.4% starch and 34.6% NDF on a DM basis. Twelve to 24 h post-calving cows also received an oral non-steroidal anti-inflammatory drug (NSAID; 1 mL/15 kg BW Meloxicam in carrier solution) or a placebo (1 mL/15 kg BW food dye in carrier solution). Butyrate supplementation and NSAID administration did not affect postpartum milk yield, serum inflammatory markers, BW or BCS change. However, butyrate-fed cows tended to have lower milk crude protein yield than control-fed cows (1.21 vs. 1.27 kg/d; $P = 0.06$). Cows fed butyrate also had lower plasma glucose on d 4 (64.3 vs. 70.8 mg/dL; $P = 0.04$) when previously administered the placebo drug. On d 7 butyrate cows tended to have lower plasma FFA (825 vs. 993 $\mu\text{Eq/L}$; $P = 0.07$) compared with control cows. Multiparous cows given the NSAID had lower postpartum DMI than placebo cows (16.7 vs. 19.2 kg/d; $P = 0.02$) when on control feed. Cows given the NSAID had higher plasma glucose on d 4 (70.7 vs. 64.3 mg/dL; $P = 0.02$) when fed the butyrate diet. Primiparous cows given the NSAID tended to ovulate later than placebo cows (29.6 vs. 18.7 d; $P = 0.09$) when fed butyrate. In the present study dietary butyrate supplementation and oral NSAID administration had no overall positive effects on cow performance or interval to first ovulation.

Blood Metabolomics Phenotyping of Dry Cows Reveals Potential Biomarkers for Susceptibility to Mastitis

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Subclinical mastitis (SCM) is a major disease of dairy cows. Our objective was to identify metabolic alterations in the serum of pre-SCM cows during the dry-off period that can be used for developing SCM lab and pen-side tests. A total of 145 cows were sampled. Mass spectroscopy was used to quantify serum metabolites at -8 and -4 wks pre-calving. Forty-four cows with SCM and another disease and 15 healthy (CON) cows were identified. Metabotyping of those cows revealed a total of 43 and 29 metabolites that differentiated SCM from CON ones at -8 and -4 wks, respectively. Results also showed that there were 4 lipids (LysoPC28:0, C5DC, PC38:0AA, PC36:0AA) and α -amino adipic acid and 4 phosphatidylcholines (PC36:0AA, PC36:0AE, PC40:2AA, PC38:0AA) and α -ketoglutaric acid that differentiated SCM cows from the CON ones at -8 and -4 wks, respectively. We then selected cows affected only by SCM ($n = 10$) and compared them with CON ($n=15$) cows. We identified 59 and 47 metabolites that differentiated the two groups at -8 wks and -4 wks prepartum. Among them, 4 serum metabolites (alanine, leucine, betaine, and ornithine) at -8 wks and 4 metabolites (alanine, pyruvic acid, methylmalonic acid, and lactic acid) at -4 wks were identified as metabolites that can serve as biomarkers for identifying cows susceptible to SCM. In conclusion, data showed that starting from -8 and -4 wks prepartum cows susceptible to SCM alone or SCM and another periparturient disease can be identified by a lab or pen-side test in the future. More research is needed to validate the panels of metabolites identified and develop lab and pen-side tests in the future.

Take Home Messages: Several blood metabolites have been identified by mass spectroscopy at -8 and -4 wks prepartum that can serve in the future as potential biomarkers to identify dairy cows susceptible to mastitis.

The Effect of Neomycin Inclusion in Milk Replacer on the Health, Growth, and Performance of Male Holstein Calves Preweaning

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The prophylactic use of oral antimicrobials in milk is a common practice in calf rearing that is thought to aid in preventing disease, but presents potential risks to the calf. The objective of this study was to investigate the effects of neomycin on calf health and growth performance. One hundred and sixty calves were assigned to one of three treatments: control (non-medicated milk replacer (MR)), short-term antimicrobial (20mg/kg BW neomycin in MR from d 1-14), or long-term antimicrobial (20 mg/kg BW neomycin in MR from d 1-28). Calf BW was measured weekly, and health scores, feed intakes, and the use of additional electrolytes and antimicrobials were recorded daily. Calves in the CON group experienced a higher proportion of days with diarrhea, longer bouts of diarrhea, and higher fecal scores. However, the time to reach first diarrhea and respiratory illness was not different, nor was the time to recover from respiratory illness. The time to intervention with additional electrolytes or antimicrobials was not different, nor was growth performance, feed intake, or feed conversion ratio. The defined daily dose of total antimicrobials that ST and LT calves received was higher than that of CON calves, who received no prophylactic antimicrobials. Given that there were no differences in performance parameters and no health benefits aside from reduced fecal scores in calves fed neomycin, current practices involving the use of antimicrobials on dairy and veal operations need to be considered more prudently.

Cool-Climate Adapted CDC Genotypes of Chickpeas Grown in Western Canada: Evaluation of Impact of Varieties and Processing Methods on Physicochemical, Nutritional, Molecular Structural Characteristics

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The primary objectives of this study were to: (1) evaluate the molecular structural, physicochemical, and nutritional characterization of cool-climate adapted CDC chickpeas developed in western Canada, and the impact of varieties and heat processing methods as an elective imperativeness hotspot for ruminants; (2) evaluate the effect of heat processing methods, Dry Heat, Wet Heat and Microwave Irradiation Processing Method on cool-season adapted CDC chickpeas as an alternative source for protein and energy feed for ruminant livestock. Within three varieties of CDC chickpeas; these being provided by the Crop Development Center; (3) reveal the molecular structure spectral results from chickpeas varieties grown in Western Canada and the molecular structure changes when heat processing methods are used using vibrational molecular spectroscopy. Chickpea samples were determined for chemical composition, energy values, CNCPS carbohydrate fractions. Subsequently, chickpea samples were incubated in the rumen for NDF degradation kinetics analysis. Later, carbohydrate related spectral features after incubation was performed results were obtained using ATR-FTIR spectroscopy.

Effect of Supplemental Selenium Source on Dairy Cow Performance, Antioxidant Status and Apparent Absorption and Retention

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The objective of this experiment was to determine how source of selenium (Se) affects animal performance, antioxidant status and apparent absorption and retention. Multiparous Holstein cows (n=24; 597 ± 49 kg BW) were blocked by days in milk (161 ± 18) and randomly assigned to receive 0.3 ppm of either: 1) organic Se (selenized yeast; **ORG**); or, 2) inorganic Se (sodium selenite; **INO**) premix, top dressed and mixed into a ration daily. Following an 11-week adaptation period (blood and milk sampled monthly) cows received an intraruminal isotope dose of Se⁷⁷ (same chemical form as premix) followed by a 4-day period of blood and rumen fluid sampling, and total collection of feces, urine, and milk. Data was analyzed with PROC GLIMMIX in SAS with fixed effects of treatment, time, and their interaction, and random effects of block and cow. Daily DMI (23 ± 0.6 kg), milk yield (35 ± 1.2 kg), plasma glutathione peroxidase (64 ± 4.2 U), and serum Se (0.11 ± 0.003 µg/g) were not different ($P > 0.1$) between treatments during adaptation period. Serum Se⁷⁷ maximum concentration (C_{max}) and area under the curve (AUC) were not different ($P > 0.1$) between treatments for 72 hours following infusion, but rumen fluid Se⁷⁷ AUC was higher ($P = 0.02$) for ORG cows. Apparent absorption (64 ± 1.3%) and retention (44 ± 1.5%) of the Se⁷⁷ dose were not different ($P > 0.1$) between treatments. Fecal excretion of the Se⁷⁷ dose was not different ($P > 0.1$) between treatments (36 ± 1.4%), but ORG cows had lower ($P < 0.01$) urinary excretion (13 ± 0.6% vs 17 ± 0.6%) and higher ($P < 0.01$) milk excretion (6 ± 0.3% vs 2 ± 0.3%) compared to INO cows. These results indicate that ORG Se improved Se content of milk and decreased Se excretion into the environment, but Se status of the cow was not affected by Se source at this supplementation level.

Synchrotron-based Study to Determine the Inherent Molecular Structure Changes Induced by Steam Pressure Times in Faba Bean Seeds

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Synchrotron technology is currently a valuable instrument to examine detailed intrinsic molecular features in a variety of materials including feed ingredients. Heat processing methods commonly used in the feed industry alter the physicochemical structure of feeds, modifying their degradation behavior when fed to livestock animals. Traditional research tools are unable to detect processing induced molecular structure changes associated to nutrient supply. Hence, this study aimed to determine the extent of protein molecular structure modifications related to steam pressure processing times in faba bean seeds. Analyzed samples belong to CDC Snowbird variety heated at 121 °C for 0, 30, 60, 90, and 120 min. SAS software 9.4 (SAS Institute, Inc., Cary, NC, US) was used for statistical analysis with significance declared at $P < 0.05$. Results showed variations in the spectra protein related areas and heights with a lower amide I area ($P < 0.01$) found at 0 min (47.53 AU, infrared absorbance units) compared to 30 min (54.16 AU) and 120 min (57.01 AU). Amide II area was higher ($P = 0.01$) at 30 min (24.36 AU) compared to 60 and 90 min (22.06 and 22.16 AU, respectively). The α -helix to β -sheet ratio was higher ($P < 0.01$) at 0 min (1.09 AU) compared to all heating times (avg. 1.01 AU). Protein molecular structure changes associated with heat processing can be directly identified using synchrotron technology. The increased knowledge in the area of feed molecular structure will benefit the application of precise dairy feeding strategies as we get a better understanding of the close relationship between individual inherent structure characteristics of each feed nutrient and the overall degradation behaviors of feedstuffs when fed to cattle.

Comparing the Performance of Timed-AI versus Automated Activity Monitors in Dairy Heifer Reproduction

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In total, 340 Holstein dairy heifers from a commercial farm near Edmonton, AB, were fitted with an ear tag activity monitor (SCR eSense, Allflex) once they were eligible for breeding (~13.5 months of age). Heifers were divided into two treatments, those in the timed-AI (TAI) treatment (n =170) were submitted to a modified 5-d Cosynch+PRID protocol (without the initial GnRH and a single prostaglandin injection at PRID removal), with the first AI scheduled for first day of breeding (D0). Those heifers in the automated activity monitor (AAM) treatment (n = 170) were bred based on activity alert, becoming eligible for AI on D0. All heifers received sexed semen for the first AI and conventional semen for subsequent breedings. Pregnancy diagnosis was done at 25, 30 d and confirmed at 45 d post AI and heifers had 3 opportunities to become pregnant to AI. Heifers in the TAI group determined non-pregnant 25 d post-AI were resynchronized and TAI 8 d later (inter-breeding interval of 33 d). There was no difference in overall pregnancy (90 vs. 94%, $P = 0.16$) and days open (25 vs. 24 d, $P = 0.95$) between TAI and AAM treatments. However, number of AI was greater in the TAI compared with AAM treatment (1.67 vs. 1.46 AI, $P = 0.01$). There was no difference in pregnancy loss or days to AI between treatment for any AI number; however, pregnancy at 30 (57 vs. 71%, $P = 0.01$) and 45 d (53 vs. 65%, $P = 0.04$) after first AI was reduced in the TAI compared with the AAM treatment.

Take Home Messages: There was no difference in the overall pregnancy and days open between TAI and AAM programs in dairy heifers. However, pregnancy at the first AI was increased in the AAM treatment. Producers who use more expensive and less fertile sexed semen for the first AI in heifers may benefit from using an AAM program compared with TAI. Our group is currently conducting an economic analysis between the TAI and AAM treatments to help inform decisions between reproductive programs in dairy heifers.

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Macronutrient Composition of Whole Milk Powder and High Fat Milk Replacer Influences Gastrointestinal Development of Preweaned Dairy Calves

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The objective of the current study was to compare the effect of whole bovine milk or MR with similar macronutrient inclusion on gastrointestinal structure and function. Eighteen male Holstein calves (46.6 ± 1.2 kg; 1.78 ± 0.13 d of age) were individually housed and randomly assigned to three times daily feeding of 3.0 L (135g/L) of either: 1) whole milk powder (WM, 26.0% fat, 24.5% protein, 38.0% lactose, n = 9); or 2) MR with high fat content (MR, 25% fat, 22.5% protein, 38.1% lactose, n = 9). Bodyweight (BW) was measured weekly and feed intake was recorded daily. On day 21 intestinal permeability was evaluated using Cr-EDTA. Calves were euthanized at 4 wk of age to obtain organ weights and intestinal samples to assess gastrointestinal structure via histological analysis. Data was analyzed in SAS software using Proc GLIMMIX for repeated measures and dissection parameters using BW as a covariate. Weekly intake of MR and BW did not differ ($P > 0.05$) and BW prior to dissection was 68.9 ± 1.4 kg. Whole forestomach, rumen, reticulum and omasum weights were 22% ($P = 0.02$), 23% ($P = 0.05$), 31% ($P = 0.05$) and 36% ($P = 0.02$) larger in WM vs MR calves, respectively. Duodenal and ileal weights did not differ between treatments; however, distal jejunum weight and whole small intestine weight were 19% ($P = 0.01$) and 15% ($P = 0.03$) greater in WM calves compared to MR calves, respectively. There were no differences in muscularis thickness, villus height or width, crypt depth or width between treatments in any tissues. Surface area of the duodenum and ileum did not vary between groups. Yet, surface area of the distal jejunum was 33% ($P = 0.03$) greater in WM than MR calves. Intestinal permeability measured as %Cr recovered in urine was greater in WM fed calves (9.5% vs 7.6%, $P = 0.08$). Overall, the results suggest that differences macronutrient composition between MR and WM affect gut mass, structure and permeability, while no affects were observed on growth or feed efficiency.

Effect of Intensity of Spontaneous Estrus Captured by Automated Activity Monitors on Ovulation Interval in Holstein Heifers

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Estrous expression is associated with the timing to ovulation and pregnancy per artificial insemination in lactating dairy cows. It is possible that the same associations exist in heifers, which would pose new questions of physiology, genetics, and environmental factors that could explain this relationship. The goal of our study was to investigate the relationship between estrus intensity, defined as the peak and duration of estrus activity, detected by an automated activity monitor (AAM), and timing to ovulation in dairy heifers. Animals were fitted with a neck-mounted accelerometer and the adjoining software was monitored twice daily for activity alerts. Upon alert, and every 8 h after until ovulation, the ovaries of the heifers were scanned by ultrasonography for the presence and subsequent disappearance of a dominant follicle (ovulation). Activity data recorded from the AAM provided an index, which defined estrus onset, peak, and end, and duration of estrus was reported in 2 h intervals. A total of 214 estrus events were recorded from 140 heifers. Peak and duration were correlated ($r = 0.61$, $P < 0.001$). The mean (\pm SD) interval from estrus onset to ovulation, duration, and peak activity was $27.94 \text{ h} \pm 6.4$, $14.87 \text{ h} \pm 4.45$, and 87.13 ± 14.61 , respectively. Duration of estrus ranged from 10.7 h to 51.3 h and was positively associated with ovulation interval ($P < 0.001$) with a predicted increase of 0.58 h in ovulation interval for every 2 h increase in duration. Peak activity was also associated with ovulation timing ($P < 0.001$) but was not as strong of a predictor as duration.

Take home messages: Estrous expression in Holstein heifers is positively associated with ovulation timing, and this may have practical implications for timing of AI and pregnancy, just as is shown in cows, which demonstrates that this relationship is not only a result of cow-related effects.

Predicting Disease in Prewaned Dairy Calves using Automated Milk Feeders

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Group housing of preweaned dairy calves is gaining popularity throughout the dairy industry. It benefits calf welfare by allowing for social interaction and increased ability to display natural behaviours. However, it is more difficult to individually monitor calves to identify disease occurrence. Automated milk feeders (AMFs) can be used to provide a higher plane of nutrition to group housed calves and also record individual feeding behaviours that could be used to predict disease in calves. The objective of this observational, retrospective, case-control study was to assess the changes in feeding behaviour exhibited by preweaned calves leading up to, during, and following the occurrence of disease. This study utilized data from two commercial dairy farms where producer treatment records were used to determine cases (calves treated for either respiratory or enteric illness). Healthy controls were selected at the end of the trial and matched to each case calf by number of days on the AMF. Differences in feeding behaviours, including milk consumption, drinking speed, rewarded visits, unrewarded visits, and total visits to the AMF were analyzed for 37 case calves and 37 control calves ($n = 74$ calves). On the 14 days surrounding the treatment (disease) event, case calves were found to consume significantly less milk (0d: 2.06 L/d, 95% CI: -2.97, -1.93L/d, $P < 0.001$) and drink slower (0d: 187.92 mL/min, 95% CI= -289.54, -86.31 mL/min, $P < 0.001$) than their healthy counterparts beginning four days prior to disease detection. Sick calves were also found to visit the feeder fewer times for an unrewarded visit and for total visits starting three days prior to and on the day of illness detection, respectively. No differences were found between sick and healthy calves with respect to rewarded visits to the AMF. The results of this study provide evidence that feeding behaviours recorded by AMF can be used to detect disease in preweaned dairy calves.

Exploring Differences in Total Digestible Dry Matter (TDDM) and Intestinal Protein Digestion (IDP) of Canola Seeds and Canola Processing Co-Products (Meals, Pellets) from Different Companies in Canada and China for Dairy Cattle

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Canola was created in the 70s as a low erucic acid and low glucosinolate seed, to produce high quality oil for human consumption and meal for use in livestock feed. China is an important user of Canadian canola products (seeds, oil, and meal). The extraction of the oil from the seed produces a co-product called canola meal. This meal is rich in protein and is used as a protein source in animal diets. However, differences in the characteristics of the seeds, or processing methods during oil extraction may affect the quality of this co-product. Plus, the synthesis of tissues and milk is related to the amino acids available to the animal for absorption in the small intestine. This study aimed to determine if there are significant differences in the intestinal digestibility (*in vitro*) of CP and DM between canola seeds and meals from different companies in Canada and to determine if there are significant differences between them in Canada and China. The three-step procedure was applied on residues from a 12-hour rumen incubation in fistulated dairy cows to estimate the intestinal digestibility of CP and DM. There were significant differences ($P < 0.05$) for TDDM (Total digestible dry matter) and IDP (intestinal digestibility of protein) of the meals between countries. The samples from China had higher TDDM (83.76% versus 81.53%, $P = 0.018$), while Canada's had higher IDP (68.51% versus 65.28%, $P = 0.016$). No significant differences were observed within countries. Based on the material analyzed during this study, it is safe to affirm that there are no significant differences in the digestibility of DM and CP between Canada and China. It was concluded that the quality of the canola seeds or meals produced in both Canada and China were similar when used in dairy rations.

Deep learning for mastitis detection and development of farm-specific detection methods

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Automated milking systems (AMS) are used in 12% of Canadian dairy farms and >20% of dairy farms in Western Canada, with indications of increasing adoption. Therefore, a need exists for accurate, early automated disease detection. AMS generate much more data than milk characteristics, and animal behavior data may offer novel indicators for disease onset, with great potential to improve mastitis detection when analyzed with state-of-the-art machine learning methods. Deep learning models were developed using data from 89 farms from across Canada using Lely AMS, of which 23 were used to validate model performance. The models were used to predict daily probability of an animal being diagnosed with clinical mastitis. Deep learning models use a series of connected neurons to identify relationships between variables, and recurrent networks capture time-dependent relationships and base predictions on individual animal patterns. Using a prediction window of 3 days, accuracy was 80% (19.6% false-positive/day and 16.5% missed cases). Furthermore, a combination of milk characteristics and behavioral traits resulted in prediction accuracy very similar to milk characteristics alone but resulted in a decrease of 5% in missed cases. Model performance was worse on farms that were not used for model training, and model performance differed considerably between the 23 validation farms. The developed model can serve as a good starting point, but farm-specific tuning would be required to reach optimal performance. The next step is going to be tuning models incrementally as new data is collected from farms and determine how much new data is needed before optimal performance is reached.

Impact. AMS data bring novel opportunities for mastitis detection. Developed models should be further tuned once implemented on new farms to optimize performance for the individual farm.

Using Synchrotron and Global Molecular Spectroscopic Techniques to Reveal Synergistic Impact and Interactive Association Between Molecular Structure and Nutrient Properties and Supply to Dairy Cows in Enzymatic and Thermal Treated Oat Endosperm Tissue and Whole Oat Grain

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As an advanced technique, synchrotron radiation-based Fourier transform Infrared microspectroscopy (SR-FTIRM) has been a rapid, direct, non-destructive and non-invasive bioanalytical method. Global molecular spectroscopic techniques, for example, attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy and diffuse reflectance Fourier transformed infrared (DRIFT) spectroscopy, will be used. For this research, CDC Nasser, CDC haymaker, CDC Arborg and Summit with three consecutive years were studied. There are three treatments: Treatment 1, steam-pressure-treated for 0, 30, 60, and 90 minutes; Treatment 2, treated by an innovative fibrolytic enzyme (Ab) under 0, 0.5, 1.0, and 1.5 mL/kg level; Treatment 3, steam-pressure-treated and then treated by Ab. The objectives of this research were to (1) Compare the molecular structure spectral features of processed oat endosperm tissues at a molecular and cellular level using SR-FTIRM; (2) Compare the molecular structure spectral features of processed whole oat grains using ATR-FTIR or DRIFT; (3) Determine the chemical profiles, protein and carbohydrate fractions profiles (CNCPS 6.55), energy profiles, degradation kinetics, intestinal digestibility, microbial protein production and true nutrient supply. The expected results are to detect a synergistic impact between processing methods on induced changes in nutrient properties, to reveal the interactive association between induced molecular structure changes and nutrient properties and true nutrient supply, and to develop absorbed nutrient supply prediction equation based on processing induced molecular structure changes. This research is also to increase economic returns to oat producers and related dairy industries through efficient utilization of new feed-type or milling type of oat grains.

Comparing Natural vs. Induced Estrus in Dairy Heifers Using an Automated Activity Monitoring System

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In total, 609 dairy heifers from a commercial farm near Edmonton, AB were fitted with an ear tag activity monitor (SCR eSense, Allflex) once they were eligible for breeding (~13.5 months of age). Estrus events were recorded as either natural (n = 881) or induced (n = 388; within 2 to 5 d after prostaglandin administration). All heifers received sexed semen for the first AI and conventional semen for subsequent breedings. Pregnancy diagnosis was done at 30 d and confirmed at 45 d post AI and heifers had 6 opportunities to become pregnant to AI. The system recorded a heat index, maximum activity change, maximum rumination change and duration of heat. The distribution of onset of estrus and peak estrus times throughout the day did not differ between natural and induced estrus. In total, 70% of heifers started estrus between 8:00PM and 8:00AM and 73% of heifers hit peak estrus between 2:00AM to 2:00PM. Heat index (82 vs. 80, $P = 0.09$) and maximum rumination change (-49 vs. -46, $P = 0.06$) tended to be greater for induced vs. natural estrus, with no difference in maximum activity change or estrus length. There was no difference in pregnancy at 30 d (68 vs. 67%, $P = 0.79$) or 45 d (64 vs. 63%, $P = 0.82$) between the induced and natural estrus groups. However, there was a tendency for interaction between treatment and semen type for pregnancy at 45 d, in which pregnancy tended to be greater in the induced group for sexed compared with conventional semen (68 vs. 58%, $P = 0.09$).

Take Home Messages: There is little difference in estrus behaviour characteristics between natural and induced estrus. The increase in pregnancy using sexed semen in the induced estrus group is likely due to sexed semen being used in the first AI and conventional semen being used in subsequent AI in less fertile heifers. Based on the estrus distribution, a heifer reproduction program using an activity monitoring system and once a day breeding may have more success breeding in late morning, when a greater proportion of heifers are close to peak estrus.

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Update on Coccidiosis in Dairy Calves

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■ Take Home Messages

- ▶ Eimeria parasites are ubiquitous and are present in all dairy herds. There are 16 Eimeria species recognized to date. Although largely specific to cattle, several species can infect bison and water buffalo. At present, only *E. bovis*, *E. zuernii* and *E. alabamensis* are thought to be capable of causing significant clinical disease in cattle.
- ▶ Clinical coccidiosis affects dairy calves typically between four and twelve months of age and adult dairy cattle are typically asymptomatic carriers. Disease risk is highest in indoor systems but can also occur at pasture, particular if stocking density is high with no pasture rotation. Disease occurs when environmental contamination with the infective sporulated oocysts is allowed to build up to high levels.
- ▶ Coccidiosis typically presents as a diarrhoeal disease that can vary from acute and severe to low grade and chronic. Sub-clinical impacts on calves such as reduced growth rates and food-conversion, impaired future fertility, and a predisposition to intercurrent disease can occur but the extent of this is poorly defined.
- ▶ Coccidiosis should be thought of as a disease where diagnosis, control and treatment are all considered at the group level.
- ▶ Diagnosis is based on fecal oocyst counts of groups of calves sharing an environment. Fecal oocyst counts > 500 oocysts/gram combined with classical clinical signs are generally used for diagnosis of acute cases. However, the identification of the Eimeria species involved is important to confirm diagnosis in less typical cases and chronic diarrhoea and to assess potential sub-clinical impacts. However, many diagnostic labs do not routinely offer Eimeria species identification and improved diagnostics is an important research priority.
- ▶ Control should centre on good husbandry and hygiene to prevent the build-up of environmental contamination and the exposure of calves to high levels of oocysts before they have had time to acquire immunity. Several pharmaceutical products are available for the prevention of coccidiosis in dairy calves but careful timing and duration of treatment is required.

■ What is Bovine Coccidiosis and What is its Cause?

Coccidiosis is a disease caused by single celled protozoan parasites of the genus *Eimeria* that can affect all species of domestic ruminants (cattle, bison, sheep goats, deer). Each different livestock species is infected by a different set of *Eimeria* species that are mainly host-specific. Thirteen different species of *Eimeria* are currently recognized to infect cattle, some of which can cause severe disease, others mild disease and some no disease essentially being thought to be commensals (Figure 1). *Eimeria* species that infect cattle are largely host-specific but several species, including the major pathogens *E. bovis*, *E. zuernii* and *E. alabamensis*, can infect closely related host species such as North American and European bison and water buffalo (Dubey, 1963; Bangoura, personal communication). The life cycle of *Eimeria* is complex and is summarized in figure 2 (Blake and Tomley, 2014). Essentially, following infection by ingestion of sporulated oocysts from the environment, the single celled sporozoites are released in the calf gastrointestinal tract and infect epithelial cells lining the small or large intestinal mucosa (depending on the particular species). There are then several rounds of multiplication in which very large numbers of parasites

are produced that eventually break out of the host epithelial cells lysing them in the process. This can cause severe damage to the lining of the gut causing severe clinical signs, particularly if occurring in the large intestine. Clinical disease is typically manifested by diarrhea and can be either chronic and low grade or acute and severe (Figure 1A). In the case of dairy cattle, clinical disease is most commonly seen between four and 12 months of age (Keeton and Navarre, 2018). Disease can occur in calves indoors or outdoors but is generally a more common problem for calves during housed indoors. Although older cattle are often infected, disease is rare in adult cattle and generally a sign of other underlying disease problems.

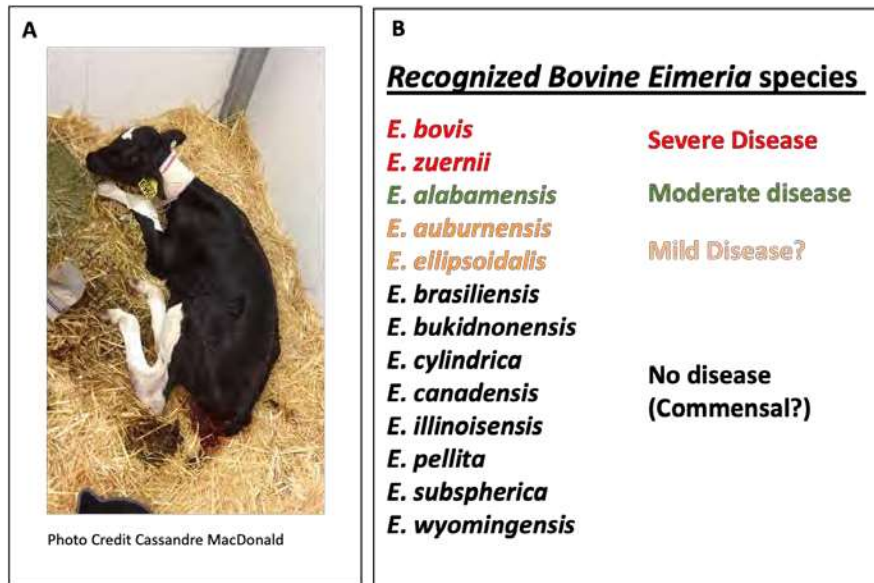
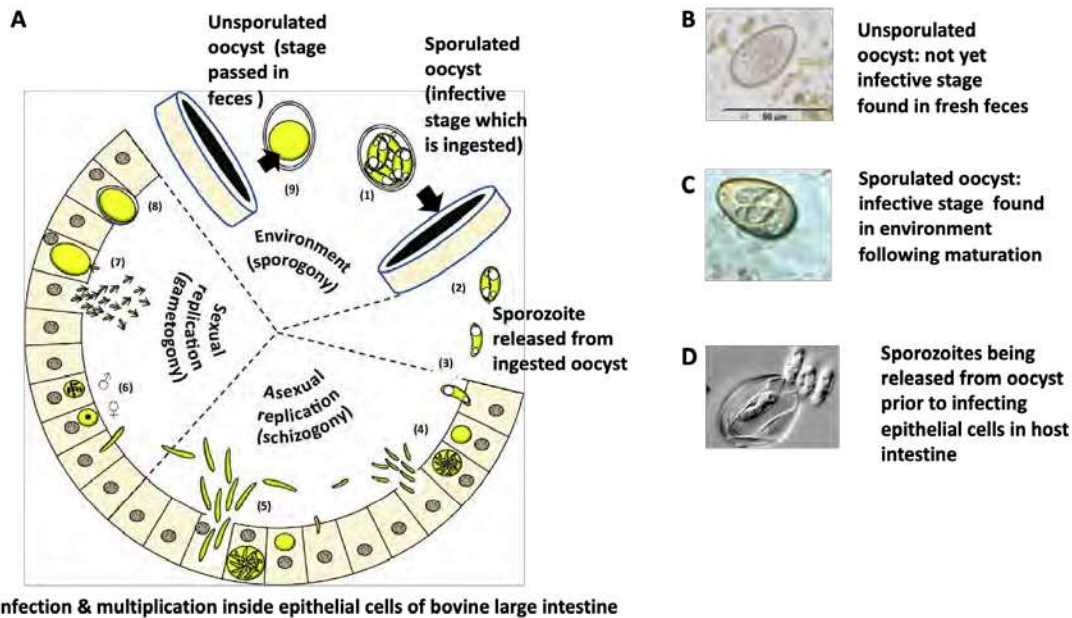


Figure 1: Panel A: Clinical coccidiosis in dairy calf. Panel B: Bovine Eimeria species



Adapted from Blake, D. and Tomley, F. (2014) Trends in Parasitology, Vol. 30, No. 1

Figure 2: Panel A. Eimeria life cycle (Adapted from Blake and Tomley, 2014). Panel B. Unsporulated oocyst. Panel C. Sporulated oocyst. Panel D. Sporozoites being released from a sporulated oocyst.

■ Why Does Bovine Coccidiosis Occur and What are the Risk Factors?

Eimeria parasites are ubiquitous and most cattle are infected at some level with different mixtures of *Eimeria* species. Transmission is by oral ingestion of the infective stages (sporulated oocysts) from the environment that are present as the result of fecal contamination by infected animals (Figure 2, Panel A). The extent to which disease is likely to occur depends on which *Eimeria* species are present and on the balance between the level of environmental contamination with sporulated oocysts and the immune response of the calf. When relatively low numbers of sporulated oocysts are ingested, parasites complete their life cycle with insufficient damage to the intestinal mucosa to cause disease. Under these circumstances, the calf gradually becomes immune to the parasite with no clinical impacts and can then withstand future challenge even with higher numbers of oocysts. On the other hand, if a calf is exposed to large numbers of sporulated oocysts in the environment before immunity has had time to develop, the developing parasites can cause serious damage to the intestinal mucosa resulting in disease. Consequently, the risk of disease depends on how rapidly the numbers of sporulated oocysts build up in the environment relative to how quickly calves develop immunity.

The capacity for *Eimeria* oocysts to build up in the environment can be understood from the lifecycle that involves several rounds of multiplication, typically two rounds of asexual and one round of sexual reproduction depending on the species, through which a single ingested sporulated oocyst can result in many millions of oocysts being shed in the feces ((Figure 2 panel A). Experimental infections have shown that more than one million oocytes of *E. zuernii* can be shed in 1 gram of bovine feces (Bangoura and Dauschies, 2007). The time between sporulated oocysts being ingested and millions of oocysts then being shed in the feces is known as the pre-patent period and can vary between 7 and 23 days depending on the particular *Eimeria* species involved (Bangoura and Bardsley, 2020). Consequently, low levels of initial environmental contamination can build up to extremely high levels relatively quickly once calves become infected. The oocytes shed in the feces into the environment are not immediately infective but need to undergo several rounds of cell division inside the oocyst (a process called sporulation) before they become infective (Figure 2, panels B and C). This can take as little as two days under optimal conditions to several

weeks depending on the temperature and relative humidity. Sporulated oocysts can remain viable and infectious for over a year and are resistant to freezing, extreme pH changes, and low oxygen but are damaged by ultraviolet light and dry conditions (Bangoura and Bardsley, 2020). Consequently, *Eimeria* oocysts build up the most rapidly in warm, moist, dark environments with high fecal contamination and minimal changes of bedding. The highest risk of disease occurs when susceptible (not yet immune) calves are kept in such conditions, particularly at high stocking densities. Once a calf has been exposed to sufficient oocysts, which may or may not cause disease as described above, it is generally solidly immune to future infection. However, immunity is specific to each *Eimeria* species and so a calf may succumb to disease several times in its life if different *Eimeria* species are involved.

■ **What are the Impacts of Bovine Coccidiosis in Dairy Calves?**

Bovine coccidiosis can occur as sub-clinical, chronic or acute disease depending on the infection level and the particular *Eimeria* species involved (discussed further below). Acute disease can present as young as two months of age but is most common in calves from four to 12 months of age as a result of the severe damage and associated inflammation of the large intestinal mucosa. Acute coccidiosis is characterised by a hemorrhagic mucoid diarrhea where fresh blood and mucus are visible in the loose feces (Figure 1, Panel A). Calves can appear to be in severe discomfort exhibiting abdominal straining and, in severe cases, can become dehydrated and may die. A more gradual build-up of oocysts can cause chronic coccidiosis that varies from low-grade diarrhea to calves simply being in poor condition or failing to grow at the expected rate. *Eimeria* infections in poultry are known to predispose to other gastrointestinal diseases such as those of bacterial origin (Collier et al., 2008; Blake and Tomley, 2014). Given the damage, inflammation and breach of integrity of the intestinal mucosa caused by *Eimeria* infections, it seems likely that similar disease associations occur in cattle although little research has been done in this area. One interesting study from Japan showed an association between hemorrhagic enteritis caused by *Clostridium perfringens* and *Eimeria zurneii* infection which is very similar to that described in poultry coccidiosis (Kirino et al., 2015).

'Nervous coccidiosis' is an unusual clinical syndrome that occurs in North American beef calves, typically in feedlots during the winter, and is characterized by neurological signs including hyperactivity and epileptiform seizures with up to 50% mortality of affected animals (Reppert and Kemp, 1972; Bangoura and Bardsley, 2020). The cause is unknown but *Eimeria*-related toxins or metabolic disturbances have been implicated. This syndrome has been reported in dairy breeds but does not appear to be common in dairy calves (Bangoura, personal communication).

Assessment of the economic impacts of bovine coccidiosis in dairy cattle is poorly researched. A 1980 study estimated that bovine coccidiosis was responsible for a ~US\$723 million loss worldwide (Lassen and Ostergaard, 2012; Bangoura and Bardsley, 2020). Losses are associated with clinical disease outbreaks including loss of production, mortalities and the costs of treating and managing affected animals as well as others in the group. Sub-clinical disease is likely to have the biggest economic impact for the industry although this is poorly defined. Such costs include those associated with exacerbation of other gastrointestinal diseases, lower feed conversion efficiency, lower growth rates and lower future fertility and milk production (Lassen and Ostergaard, 2012). Although the use of in-feed ionophores and anticoccidials currently reduces some of these potential impacts, animals with high oocyst counts and clinical disease outbreaks still occur. Furthermore, the ongoing debate regarding the prophylactic use of pharmaceuticals in food producing animals, the status of anticoccidials as antimicrobials and the trend towards more organic systems are all likely to increase the importance of sub-clinical and clinical coccidiosis in dairy calves over the coming years.

■ **How is Bovine Coccidiosis Diagnosed?**

Coccidiosis is diagnosed by the detection of *Eimeria* oocytes in fresh feces using fecal floatation methods, similar to those used to detect roundworm eggs (Figure 2B). Diagnosis should be made on a group basis because oocyst counts can vary widely between individual calves within a group (Figure 3). Consequently,

samples should be taken from multiple animals in the group (preferably ten or more) including those showing no clinical signs and affected animals.

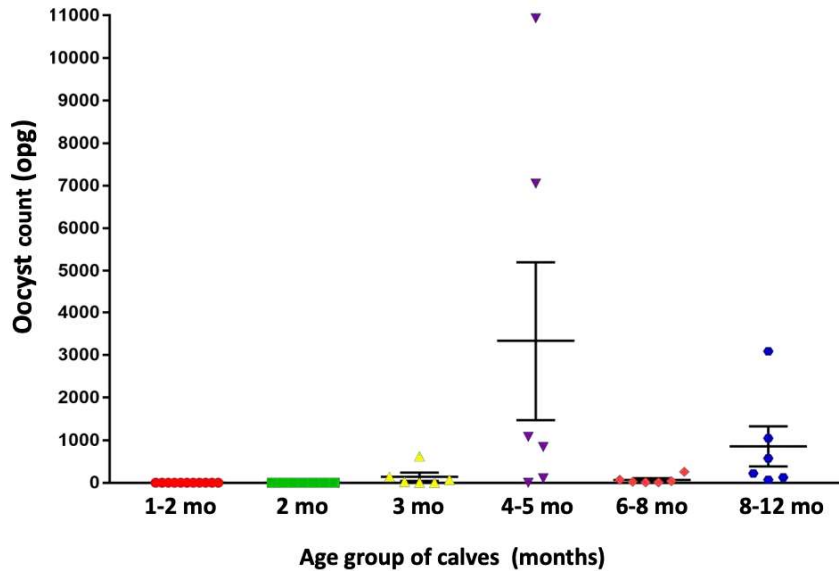


Figure 3: Eimeria oocyst counts in fecal samples taken from individual dairy calves from a farm in western Canada. Each age group was kept in separate pens. opg = oocysts per gram. No clinical signs were apparent in any of the calves.

Interpretation of fecal oocyst counts can be difficult, particularly for chronic disease or when assessing the risk of sub-clinical production impacts. Although there are 13 recognized *Eimeria* species that can infect domestic cattle, only two are considered to cause severe disease (*E. bovis* and *E. zuernii*), most commonly affecting housed calves, and one causing moderate disease (*E. alabamensis*) that can also cause problems in calves at pasture (Figure 1B). Two other species are considered to be potential mild pathogens (*E. ellipsoidalis* and *E. auburnensis*) with the rest assumed to be non-pathogenic and essentially commensal. However, there has been limited research into many of these *Eimeria* species and so their true potential for clinical impacts or predisposition to other diseases is still poorly understood. Infections in dairy calves are typically mixtures of pathogenic and presumed non-pathogenic strains (Figure 4). Consequently, high fecal oocyst counts do not necessarily suggest an imminent disease risk if they mainly comprise presumed non-pathogenic species although such counts should be taken as a flag that housing, husbandry and hygiene procedure are conducive to a build-up of *Eimeria* oocysts in the environment. Consequently, it is critical, both for clinical diagnosis and for routine monitoring, to take into account which species are present and in what proportions. For cattle, counts > 500 oocysts per gram of feces of one or more pathogenic species are potentially clinically significant (Joachim et al., 2018). Even in the absence of clinical signs, finding counts of > 500 oocysts of pathogenic *Eimeria* species in several animals in a group should raise concerns of a potential imminent disease risk. Lower counts of several 100 oocysts per gram of pathogenic species are warnings that further monitoring and preventive measures are likely required. In the example provided in Figure 4 for Pen 1, an oocyst count of > 2000 opg comprising mainly *E. alabamensis* could result in mild diarrheal disease or mild negative impacts on growth. Although the oocyst counts in pens 3 and 4 are below 500 opg they comprise mainly *Eimeria bovis*, which is a severe pathogen, and so there is potential for more severe disease or growth impacts to occur if counts rise and so careful monitoring is recommended.

The determination of the species of *Eimeria* present is traditionally achieved by detailed microscopic examination of oocysts following their sporulation in the lab. However, this is a highly specialized and

extremely time-consuming technique and not routinely performed in many diagnostics labs. Consequently, diagnosis is often presumptive based on total oocyst counts and there is relatively little surveillance data available on the prevalence and infection intensities of the different bovine *Eimeria* species in Canadian dairy cattle. We are currently undertaking research to develop molecular diagnostic methods, based on polymerase chain reaction and next-generation amplicon sequencing methods for both surveillance and diagnostics similar to approaches we have previously applied to parasitic nematodes of cattle (Avramenko et al., 2015, 2017). These approaches have great potential to improve the accuracy and scalability of species-specific diagnostics tests in bovine coccidiosis.

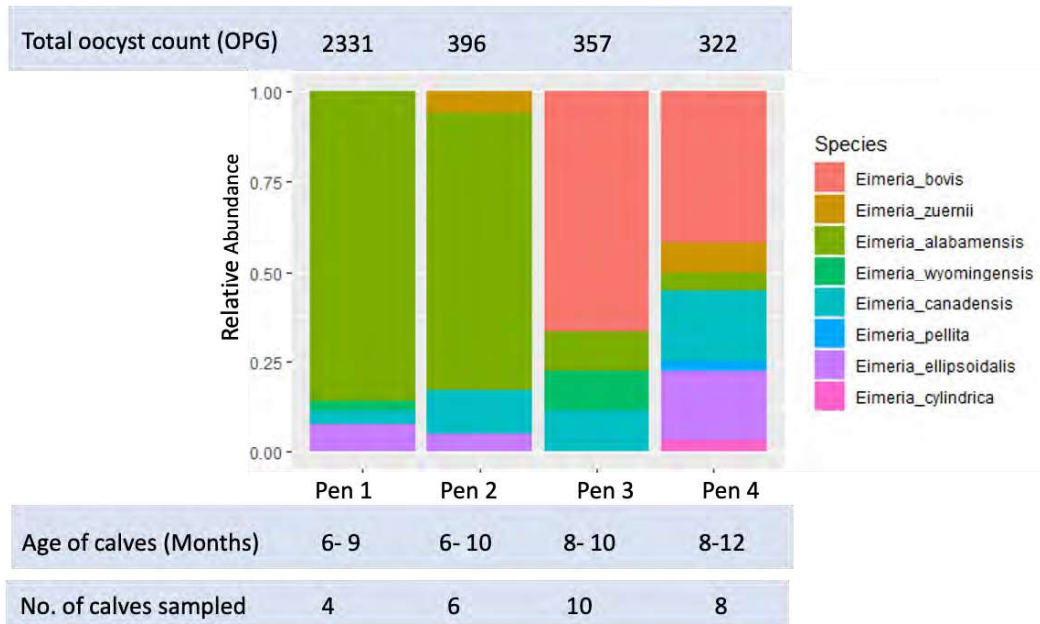


Figure 4: Eimeria oocyst counts and species proportions based on morphology of 100 sporulated oocysts following harvesting from pooled fecal samples from 4 different pens of calves from the same western Canadian dairy farm. Credits: Thanks to Dr. Berit Bangoura and Dr. Rao Parimasetti for the morphological identification and data analysis.

▪ How is Coccidiosis Prevented?

Eimeria parasites are ubiquitous in calves and routine fecal examination will generally detect oocysts present in some individuals, particularly in calves housed indoors. It is practically impossible to achieve an 'Eimeria-free' environment in the standard husbandry conditions used for either beef or dairy cattle. Indeed, it may be undesirable to eradicate all *Eimeria* species because some may be part of the normal commensal gut flora. Instead, the aim should be to minimize the build-up of sporulated oocysts in the environment and allow the gradual acquisition of immunity to reduce the risk of disease outbreaks or sub-clinical production loss. The most important control measures are good hygiene and husbandry practices including ensuring good colostrum intake, sufficient clean bedding with timely removal, prevention of fecal soiling of food troughs and water sources and separating groups of calves by age with all-in all-out systems using good disinfection and cleaning between batches of calves. Bleach-based disinfectants are partially, but not completely, effective at destroying *Eimeria* oocysts, which are highly resistant and long lasting in the environment (up to over a year; Bangoura and Bardsley, 2020). Consequently, physical removal of bedding with thorough removal of debris (e.g., steam cleaning) is important, particularly in herds with a history of problems. For calves on pasture, a proportion of oocysts will survive the winter on pastures and so annual rotation of grazing should be considered if stocking densities are high or there is a history of disease. Routine monitoring of calves to determine oocyst counts can be a valuable tool to assess disease risk and

the effectiveness of management practices, particularly for herds with a history of disease problems. As discussed earlier, it is important to test multiple animals in a group (ideally 10 to 20 calves) because there is a high degree of variance in the oocyst shedding of individual animals (Figure 3). Fecal samples taken from individual calves, either fresh from the bedding or per rectum, can be pooled in the lab and a group oocyst count determined to save on diagnostic costs.

There are currently no vaccines available for bovine coccidiosis, but several pharmaceutical compounds can be used in prevention (Noack et al, 2019). In Canada the pharmaceuticals for bovine coccidiosis prevention are typically administered as feed additives over a period of several weeks (Figure 5). These fall into two broad categories: coccidiostatic compounds that prevent parasite multiplication and allow the calf immune response to kill the parasites (amprolium, decoquinate, and sulfonamides) and coccidiocidal compounds that directly kill the parasites (either ionophores such as monensin and lasalocid or the symmetric triazine, toltrazuril). These different drugs have different modes of action and act on different stages of the parasite life cycle (Noack et al, 2019). Those that target the early stages (sporozoites and merozoites) are typically used for prevention (as opposed to treatment) whereas those that target multiple stages can be used for both prevention and treatment (Figure 5). The principal of control is essentially one of metaphylaxis rather than prophylaxis; it is better that animals are already infected with *Eimeria* before the drug administration is initiated to allow immunity to develop before infection levels reach a point where there is intestinal damage. All animals in the group need to be treated to reduce overall environmental contamination. This can be achieved in weaned animals by putting drugs such as lasalocid, monensin or decoquinate in the feed or in the water supply for at least 28 days. Shorter treatment periods run the risk of simply delaying parasite development, leading to disease outbreaks in older animals. Decoquinate is preferred in younger calves (less than four months of age) because there is a toxicity risk with ionophores such as monensin in this age group (Ensley, 2020). Another strategy is to use toltrazuril, which is different to the other drugs in that it is given as a single oral dose. The drug is highly effective against all the different internal stages of the parasite and is used strategically as a single dose timed several weeks before the major risk period occurs, i.e., during the pre-patent period. The aim is to have allowed the infection to progress sufficiently to allow immunity to develop but break the lifecycle before disease and larger levels of environmental contamination occur. Little research has yet been conducted on drug resistance in bovine *Eimeria*. However, given how commonly it occurs in poultry *Eimeria* species to most classes of anticoccidials it seems likely it will be present in bovine *Eimeria* (Noack et al., 2019). One recent case report of interest is the development of toltrazuril resistance in *Eimeria* in sheep in Norway (Odden et al., 2018).

■ **How are Clinical Coccidiosis Cases and Outbreaks Managed?**

The focus should always be on prevention as opposed to treatment of clinical disease because once clinical signs are apparent significant intestinal damage has already occurred. Severely affected animals are a mortality risk and may only partially recover having subsequent poor growth and fertility due to long term damage of the gastrointestinal tract. When disease outbreaks occur, coccidiosis should be thought of as a group or herd disease. If one, or several, calves in a group show clinical signs, then others in the same group sharing the environment will be exposed to the same infection levels and so are both at risk of disease and are also contributors to environmental contamination. Consequently, all animals in a group should be treated, not just those with clinical signs. Several drugs are available for treating clinical cases in Canada with the most commonly used being amprolium or toltrazuril (Figure 5). Toltrazuril is effective against all the different parasite stages inside the host and treatment can reduce the severity and duration of diarrhea in clinical cases and a significant reduction in oocyst shedding of treated animals. Treating the whole group early, when only a few individuals are showing clinical signs, may prevent clinical disease in those individuals still in the pre-patent phases of infection. It is also important to remove the animals from the contaminated environment or, if not possible, to clean the environment of contaminated bedding to prevent further infection. Clinically affected individuals should ideally be separated from the rest of the group and given supportive oral and parenteral fluid therapy as necessary. The density of the remaining animals in the pens should be reduced if at all possible.

	<u>Parasite Stage Targeted</u>	<u>Route of administration</u>	<u>Primary Use</u>
Lasalocid (Bovatec, Avatech)	Sporozoites & merozoites	In-feed	Prevention
Monensin (Rumensin, Coban)	Sporozoites & merozoites	In -feed	Prevention
Decoquinatate (Deccox)	Sporozoites	In- Feed or milk	Prevention
Amprolium (Amprol, Apromed)	2nd generation Schizonts	In-feed or water	Mainly Treatment
Toltrazuril (Baycox)	All intracellular stages	Oral suspension (single dose)	Prevention and Treatment

Figure 5: Pharmaceutical agents licensed for bovine coccidiosis control in Canada

■ Future Directions and Research Priorities

There has been remarkably little research on bovine coccidiosis relative to its importance. In many cases, control is largely achieved by the inclusion of ionophores or other anticoccidial agents in calf rations or water supplies. Disease problems are only likely to increase in the future as trends to move away from the prophylactic use of pharmaceuticals in food producing animals continue. There are many research questions and tools that are needed in this area including the following:

Some Required Research and Control Tools

- Improved tools for routine diagnostics and surveillance
- Vaccines against the most pathogenic bovine Eimeria species

Some Key Research Questions

- What are the prevalence and infection intensities of the different Eimeria species in different age groups, production systems and geographical areas?
- What is the pathogenicity of the less studied bovine Eimeria species?
- What is the extent of sub-clinical impacts of Eimeria infections in dairy calves?
- To what extent does Eimeria infection predispose to, or exacerbate, other gastrointestinal diseases in dairy calves?
- How do Eimeria communities impact the dairy calf microbiome and what role does this play in health and disease?

- To what extent is drug resistance developing to the different anticoccidial compounds in bovine *Eimeria* species?

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Impact of Voluntary Waiting Period and First-Service Management Strategies on the Reproductive Performance and Profitability of Dairy Cows

Julio O. Giordano

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■ Take Home Messages

- ▶ Extending the duration of the voluntary waiting period (VWP) from 50 or 60 days in milk (DIM) to 88 DIM can increase first service pregnancy per AI (P/AI) but delay overall time to pregnancy when either all timed AI (TAI) or combined (AI in estrus and TAI) programs are used for first service.
- ▶ The greatest positive effect of extending the VWP on first service P/AI may be observed in primiparous cows.
- ▶ Delaying first service from 60 to 88 DIM may result in greater profitability for primiparous, but not for multiparous cows.
- ▶ Changes in VWP duration affected profitability primarily by differences in replacement cost, and to a lesser extent, by income over feed cost.
- ▶ In general, the effect of manipulating the duration of the VWP on herd performance and economics depends upon complex interactions between reproductive performance, herd exit dynamics, lactation performance, and economic conditions.

■ Introduction

Timing of pregnancy during lactation affects dairy herd profitability by defining the calving interval, milk production efficiency, and herd replacement dynamics. Insemination and conception risk after the end of the voluntary waiting period (VWP) are the two major determinants of time to pregnancy during lactation. The duration of the VWP may also influence timing of pregnancy because it determines when cows become eligible for insemination.

Dairy farms in the United States traditionally begin inseminating cows at approximately 40 to 50 days in milk (DIM) because sub-optimal detection of estrus and fertility to AI required that cows received multiple services to conceive. In recent years, however, better cow health and reproductive management programs that ensure inseminating cows by a set DIM led to increases in reproductive and productive performance of well-managed dairy herds. Improved detection of estrus and fertility reduces the number of inseminations needed to conceive and the variation in the timing required for cows to become pregnant. Thus, there is an opportunity to better control timing of pregnancy during lactation and thereby maximize profitability. In this regard, extending the VWP by a reasonable amount of time (20 to 30 days) may be a simple and inexpensive change with potential to impact the profitability of dairy herds.

Despite the potential effect of manipulating the duration of the VWP on herd reproductive performance and profitability, very limited data are available about the reproductive performance and the profitability of dairy cows managed with different VWP duration. Indeed, in recent years many dairy farms have extended the VWP for their cows without a clear understanding of the implications to herd performance and profitability.

Potential Effects of VWP on Physiological Status of Cows Before First Service

Extending the duration of the VWP may improve reproductive performance of cows by multiple mechanisms. For example, it may provide more time to recover uterine health (Gilbert et al., 2005; Sheldon et al., 2009) by improved immune status later in lactation, or more time to resolve inflammation after calving, or both (LeBlanc et al., 2011; 2014). Likewise, a longer VWP may provide cows more time to resume ovarian cyclicity (Butler, 2003) allowing more estrous cycles before first service, which has been linked to fewer days to first service and greater pregnancies per AI (P/AI; Thatcher and Wilcox, 1973; Butler and Smith, 1989). Delaying first service beyond the period of negative energy balance nadir in early lactation also may improve reproductive performance by avoiding insemination during negative energy balance. Optimum body condition score (BCS) at the time of insemination is strongly associated with high probability of pregnancy (Souza et al., 2007; 2008; Carvalho et al., 2014).

Previous Research on VWP Duration and Reproductive Performance

Despite the potential benefits of extended VWP on reproductive performance, the impact of this management strategy on overall herd performance has not been fully elucidated. Few randomized controlled experiments have evaluated the implications of VWP duration on the reproductive performance, herd exit dynamics, and economics of dairy cows. In an experiment with a limited number of second-lactation cows (i.e., 54 cows per group), Van Amburgh et al. (1997) found no differences in P/AI at first service, heat detection efficiency, and services per conception when comparing VWP of 60 versus 150 days. Furthermore, Arbel et al. (2001) observed no effect of extending VWP by 60 days (from 90 to 150 days in primiparous cows and from 60 to 120 days in multiparous cows) on reproductive performance of dairy cows under Israeli conditions. In this previous experiment, only cows with above-average milk production that did not calve during summer were included. In contrast, in an experiment conducted in Germany using only cows with above- or below-average milk production, an increment of 13 and 20 percentage points in P/AI to first service was observed when VWP was extended from 77 to 98 or from 56 to 77 DIM for high- and low -producing cows, respectively (Tenhagen et al., 2003).

More recently, Gobikrushanth et al. (2014) reported the results of a retrospective cohort study using data from a commercial farm in Florida that extended VWP duration during summer only. Cows with the extended VWP had improved first service P/AI, but more days open, and longer calving intervals. Results from the previous study might have been confounded by season of AI because cows with short VWP (57 to 63 days) received first service during summer and fall, whereas cows with long VWP (64 to 121 days) received first service during fall only. Moreover, the reproductive program used to submit cows for first service resulted in overlapped DIM at first service for a substantial proportion of cows.

Collectively, the ambiguous results and multiple exclusion criteria of these previous studies did not allow a decisive conclusion that extending the duration of the VWP is beneficial for the reproductive and lactation performance of dairy cows or the determination of the potential effects of extending the VWP on the herd exit dynamics and economics of dairy herds.

■ Recent Research on Duration of the VWP

Extending VWP from 60 to 88 DIM and Using All Timed AI for First Service

We recently conducted an experiment to evaluate the reproductive performance, herd exit dynamics, and economics of dairy cows managed with a VWP of 60 or 88 DIM in commercial dairy farms. We also were interested in evaluating the effect of longer VWP on markers of physiological and energy status before first service. Based on expected physiological benefits of delaying first service (i.e., improved uterine health, reduced rate of anovulation, improved BCS, and reduced systemic inflammation), we hypothesized that extending VWP duration from 60 to 88 DIM would increase P/AI to first service and improve overall reproductive performance (i.e., reduce overall time to pregnancy after calving).

Cows from three commercial farms in New York were blocked by parity (primiparous vs. multiparous) and the multiparous cows were stratified by total milk yield recorded for the previous lactation. Thereafter, cows were randomly assigned to a VWP of 60 [VWP60; $n = 1,265$] or 88 [VWP88; $n = 1,260$] DIM. For first service, all cows received the Double-Ovsynch (DO) protocol (GnRH-7 days-PGF-3 days-GnRH-7 days-GnRH-7 days-PGF-56 h-GnRH-16 to 20 h-TAI; Souza et al., 2008) for synchronization of ovulation. For second and greater AI services, cows were submitted for insemination after detection of estrus. At all three farms, cows not detected in estrus and re-inseminated before non-pregnancy diagnosis at 39 ± 3 days after AI received TAI after resynchronization of ovulation with the Ovsynch protocol (GnRH-7 days-PGF-56 h-GnRH-16 to 20 h-TAI) initiated 32 ± 3 days after AI (D32-Resynch).

All farms housed cows in free-stall barns with four or six rows of stalls, milked cows three times or twice daily (one farm), and cows were supplemented with recombinant bovine somatotropin (rbST; Sometribove zinc, Posilac, Elanco Animal Health, Indianapolis, IN).

Physiological Traits before First Service

Our results for multiple markers of physiological status supported the hypothesis that a longer VWP would lead to an improved uterine environment, reduced anovulation, improved BCS, and reduced systemic inflammation before first service. The effect of extending VWP duration on uterine health was evident because fewer cows had purulent vaginal discharge (PVD) and cytological endometritis (CYTO) at the beginning of DO and at 10 days before TAI. The longer VWP also resulted in more cows with a BCS ≥ 2.75 , which has been associated with greater first service P/AI. Assuming that most cows lost body reserves after calving, our results (data not shown) indicated that the longer interval from calving to first service for cows in the VWP88 treatment allowed recovery of more body reserves. Collectively, these observations for physiological markers and overall metabolic status indicated that providing cows more time to recover before first service was a feasible strategy to promote a physiological status more conducive to pregnancy.

A greater proportion of cyclic cows at the beginning of the DO protocol in the VWP88 group also reflected the effect of additional time for resumption of cyclicity, whereas the similar proportion of cyclic cows observed 10 days before TAI in both groups reflected the efficacy of the DO protocol to resolve anovulation in cows exposed to the VWP60 treatment. This was expected because previous studies have demonstrated that GnRH-based presynchronization protocols are effective for reducing the proportion of anovular cows before TAI (Souza et al., 2008; Herlihy et al., 2012).

Reproductive Outcomes

In support of our main hypothesis, extending the duration of the VWP from 60 to 88 DIM after synchronization of ovulation with the DO protocol increased P/AI after first service in lactating dairy cows (Table 1). Nevertheless, most of the observed difference was attributed to the greater P/AI of primiparous cows in the VWP88 treatment (no significant difference for multiparous cows). The reason for the different response to treatments by parity is unclear because both groups presented a fairly similar physiological response to the extension of the VWP. Differences between parities in metabolic status, health, or both not captured by the traits monitored in this experiment may explain such a discrepancy. As expected, P/AI was greater ($P < 0.01$) for primiparous than multiparous cows (50.4 vs. 38.0%, respectively) and cows with low (45.7%) and medium (46.7%) accumulated milk production up to 30 DIM had greater ($P = 0.02$) P/AI than cows with greater (39.9%) milk yield.

A lack of difference in pregnancy loss, proportion of cows inseminated after a detected estrus, and P/AI for second and greater services suggested that extending the duration of the VWP should not be expected to reduce pregnancy losses for cows pregnant after first service, improve the likelihood of re-insemination to estrus, or the fertility to second and greater AI services. Conversely, extending the VWP from 60 to 88 DIM, affected the total number of AI services up to 350 DIM because cows in the VWP60 treatment had more ($P = 0.04$) inseminations than cows in the VWP88 treatment (2.6 vs 2.4 services, respectively). This was the

result of earlier opportunities for re-inseminations in cows not pregnant to previous AI services in the VWP60 group.

Table 1. Effect of extending duration of the voluntary waiting period from 60 to 88 DIM on pregnancies per AI and pregnancy loss after first service TAI in lactating dairy cows

Item ^{1,2}	P/AI 39 days after timed AI ³	P/AI at pregnancy confirmation ⁴	Pregnancy loss ⁵
Primiparous	----- % (n/n) -----		
VWP60	46.2 ^a (214/463)	44.0 ^a (201/457)	3.4 (7/208)
VWP88	55.0 ^b (249/453)	52.3 ^b (237/453)	4.8 (12/249)
Multiparous			
VWP60	36.2 (263/726)	33.1 (237/717)	6.7 (17/254)
VWP88	40.1 (280/698)	36.3 (250/689)	7.8 (21/271)
All parities			
VWP60	40.1 (477/1,189)	37.3 (438/1,174)	5.2 (24/462)
VWP88	46.0 (529/1,151)	42.6 (487/1,142)	6.4 (33/520)
P-value			
Treatment	<0.01	<0.01	0.36
Parity	<0.01	<0.01	0.04
Treatment x parity	0.19	0.22	0.68

^{a-b}Means with different superscript letters within parity and pregnancy outcome differ ($P \leq 0.05$).

¹VWP60 = first service timed AI at 60 ± 3 DIM after the Double-Ovsynch protocol.

²VWP88 = first service timed AI at 88 ± 3 DIM after the Double-Ovsynch protocol.

³P/AI = pregnancies per artificial insemination.

⁴Reconfirmation of pregnancy status in pregnant cows was conducted by transrectal palpation at 67 ± 3 days on one farm or transrectal ultrasonography at 95 ± 3 and 109 ± 3 days after AI on the other two farms.

⁵Discrepancy in number of cows for calculation of P/AI at different time points and pregnancy loss due to cows leaving the herd because of sale or death before pregnancy reconfirmation.

Our overall results for P/AI and re-insemination dynamics are in agreement with previous studies, which showed improved P/AI after extending the duration of the VWP (Tenhagen et al., 2003; Gobikrushanth et al., 2014). Nevertheless, direct comparisons between studies are difficult because of differences in experimental design and interactions between treatments and other confounders. Thus, despite substantial variation across studies, the collective results of the current experiment and others (Tenhagen et al., 2003; Gobikrushanth et al., 2014) conducted under conditions more similar to ours (i.e., using TAI and less difference in VWP duration) indicate that extending VWP duration increases P/AI to first service. The magnitude of the increment in P/AI, however, may be affected by parity, method of insemination, season, milk yield level, and the magnitude and timing of the extension of the VWP.

Because timing of pregnancy for lactating dairy cows is determined by the combined effect of all AI services rather than first service only, evaluating the pattern of pregnancy creation during the entire lactation is essential to truly determine the effect of VWP duration on reproductive performance. Rather than focusing only on first service outcomes, dairy managers should consider the potential effect of manipulating the duration of the VWP on pregnancy dynamics during the entire lactation and for all cows. In this regard, cows in the VWP60 treatment in our experiment became pregnant at a faster rate after calving than cows in the VWP88 treatment regardless of parity as evidenced by a greater hazard of pregnancy (i.e., an indication of the different speed at which cows become pregnant in two groups) after calving (Figure 1). As a result, median (days at which 50% of cows were pregnant) and mean days to pregnancy were 102 and 132 days for the VWP60 treatment and 128 and 154 days for the VWP88 treatment (Figure 1). The hazard of pregnancy also was affected by parity ($P < 0.01$) because primiparous cows became pregnant at a faster rate than multiparous cows (HR 1.48, 95% CI 1.36 to 1.62). Cows with greater milk yield up to 30 DIM became pregnant at a slower rate (HR 0.84, 95% CI 0.76 to 0.94) than cows with medium milk yield (no difference between high and low milk yield). Essentially, the reduced P/AI to first service for cows in the

VWP60 treatment was fully compensated by the creation of more pregnancies at earlier DIM because of more and earlier opportunities for re-insemination.

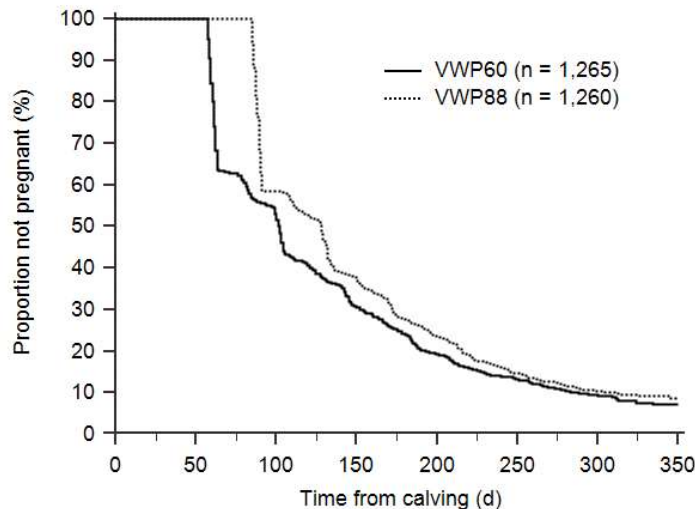


Figure 1. Survival curves for time to pregnancy after calving for cows that received a TAI at 60 (VWP60) or 88 DIM (VWP88) after the Double-Ovsynch protocol. Cows in the VWP60 treatment became pregnant at a faster ($P < 0.001$; HR 1.34; 95% CI 1.23 to 1.47) rate than cows in the VWP88 treatment

The faster rate of pregnancy creation, however, did not result in a reduced proportion of nonpregnant cows at 350 DIM ($P = 0.28$; VWP60 7.3 vs. VWP88 8.6%). These results indicate that the greatest consequence of longer VWP is shifting timing of pregnancy towards later lactation rather than generating a different proportion of pregnant cows during lactation. In agreement, two other studies reported the same patterns of pregnancy creation (Tenhagen et al., 2003; Gobikrushanth et al., 2014) as those observed in our experiment.

Herd Exit Dynamics

Cow parity and pregnancy status are major determinants of the herd exit dynamics in dairy farms. Pregnant cows and younger cows (e.g., primiparous vs. multiparous) have reduced risk of removal from the herd (De Vries et al., 2010; Pinedo et al., 2010). Indeed, in our experiment, a smaller proportion of primiparous than multiparous cows left the herd and primiparous cows had a similar herd exit dynamic regardless of VWP treatment (HR 1.12, 95% CI 0.77 to 1.61). These results reflected the protective effect for culling of early pregnancy and younger age. Conversely, for multiparous cows a greater proportion of cows from the VWP88 treatment exited the herd as lactation progressed ($P = 0.03$; HR 1.21, 95% CI 1.02 to 1.44) reflecting the compounded effect of delayed pregnancy and greater culling pressure in older cows.

We also observed that cows in the low milk-yield group had a greater hazard of culling ($P < 0.01$) than cows in the medium (HR 1.72, 95% CI 1.43 to 2.07) and high (HR 2.04, 95% CI 1.69 to 2.47) milk-yield groups. Therefore, milk yield level also played a role because nonpregnant cows with medium and high milk yield within parity had less culling pressure than cows with low milk yield. These results indicate that through its effect on timing of pregnancy during lactation, manipulating the duration of the VWP also may affect the herd exit dynamics, which may have important economic implications. As pregnancy is delayed cows, in particular multiparous cows, are more likely to leave the herd.

Economic Outcomes

We monitored cows enrolled in our experiment for 18 months after calving to determine individual cow profitability based on income over feed cost (IOFC), replacement costs, reproductive programs costs, rbST supplementation cost, operating expenses, and value of calves born. Cash flow for an 18-month period after calving for each cow enrolled in the experiment was calculated by addition of all these expenses and revenues. In order to better represent the reality of a dairy farm, we considered that every cow enrolled in the trial filled-in a slot at the dairy and the slot had to remain occupied for the entire 18-month period to maintain constant herd size. Therefore, every cow that left the herd because of sale or death was replaced by a randomly selected first lactation cow from the same experimental treatment. This cow contributed to expenses and revenues up to the end of the 18-month period (i.e., filled-in the original slot occupied by the cow it replaced). If the replacement cow left the herd before the end of the 18-month period, she also was replaced by another randomly choose first-lactation cow. The method used for our economic analysis (i.e., fixed period of time including a significant portion of the lactation following application of the experimental treatments) was meant to better represent the effect of reproductive performance on herd profitability. Otherwise, the effect of timing of pregnancy on the current and subsequent lactation is not captured. Because of substantial differences in performance and profitability between primiparous and multiparous cows, data were analyzed separately by parity group. Results for profitability by parity group are presented in Table 2.

Table 2. Effects of extending duration of the voluntary waiting period from 60 to 88 DIM on revenues and expenses during 18-mo after calving in the experimental lactation.

Item	Primiparous				Multiparous			
	VWP60 (n=480)	VWP88 (n=471)	Diff	P- value	VWP60 (n=785)	VWP88 (n=789)	Diff	P- value
Milk income over feed cost	3,806±119	3,803±119	-3	0.95	4,363±132	4,324±132	-39	0.38
Calf value	100.5±3.5	103±3.5	2.4	0.59	80.8±2.5	7.8±2.5	-2.80	0.43
Replacement cost	327.3±52.1	259±52.3	-68.3	0.07	624.9±31.4	673.6±31.3	48.7	0.16
Reproductive cost	97.6±1.4	91.3±1.4	-6.3	<0.01	104.1±2.0	93.6±2.0	-10.5	<0.01
bST cost	215.9±23.6	222±23.6	5.7	<0.01	219.4±25.6	224.9±25.6	5.5	<0.01
Other operating expenses	1,512	1,512			1,512	1,512		
Cash flow	1,756±148	1,824±148	68	0.32	2,006±124	1,921±124	-85	0.19
Cash flow per day	3.25±0.28	3.37±0.28	0.12	0.32	3.71±0.23	3.56±0.23	-0.15	0.19

¹VWP60=first service timed AI at 60±3 DIM after the Double-Ovsynch protocol.

²VWP88=first service timed AI at 88±3 DIM after the Double-Ovsynch protocol.

For primiparous cows, cash flow per slot per 18-month or per day was similar ($P = 0.32$) for the VWP60 and VWP88 treatments despite a \$68 numerical difference in favor of the VWP88 group. Most of the difference between treatments resulted from greater replacement cost for the VWP60 treatment because the small differences observed for the rest of the costs offset each other. The difference in replacement cost resulted primarily from a greater cost in the subsequent lactation because a slightly greater percentage of second lactation cows (i.e., first lactation during experimental lactation) left the herd before the end of the 18-month period. Thus, when attempting to extrapolate the results of our experiment to other farms, it is important to recognize the dominance of replacement cost over total profitability because different replacement cost dynamics (i.e., different culling pressure and different cost of culling) may be observed across herds and changing market conditions. Indeed, when we simulated the potential effect of changes

in economic conditions (i.e., milk prices, reproductive cost, heifer replacement costs, and calf values), replacement costs accounted for up to almost 80% of the total variation in profitability.

Results for multiparous cows were opposite to those of primiparous cows. Although a statistically significant difference between treatments was not observed, cows in the VWP60 were more profitable by \$85 per slot per 18-month than cows in the VWP88 treatment. In this case, however, replacement cost was greater for the VWP88 reflecting increased culling pressure in nonpregnant cows in the VWP88 treatment during later lactation. Such contrast in results for overall cash flow likely reflected differences in milk production persistency (i.e., lactation curves are less persistent for multiparous than primiparous) and the interaction between parity and risk of leaving the herd as lactation progressed (i.e., only multiparous but not primiparous cows in the VWP88 were more likely to leave the herd). Although to a lesser extent than for primiparous cows, replacement cost explained a substantial proportion of the numerical economic differences for the results with fixed economic values or when we simulated varying economic conditions.

In summary, the economic outcomes for our experiment indicate that extending the VWP from 60 to 88 DIM when using only TAI to submit cows for first service may result in greater (numerical) profitability for primiparous cows, primarily through a reduction in replacement costs. Conversely, the same extension of the VWP duration for multiparous cows may lead to economic losses (numerical) primarily from greater replacement cost and reduced income-over-feed costs, which cannot be offset by reduced reproductive program costs.

Results from our experiment should be interpreted with caution because despite the large number of cows we did not detect statistically significant differences for overall cash flow; this may be related to the fact that all cows received rbST and the particular replacement dynamics of the herds involved in our research that may have been affected by individual farm management decisions and the economic conditions during the study. Of note, the method used to calculate cow profitability also may vary depending on whether profitability per unit of time and slot, or per cow, regardless of time and herd size constraints are calculated.

Effect of Method of Submission for First Service and VWP Duration on Reproductive Performance

Dairy managers need to determine not only the duration of the VWP for their cows, but also the type of management strategy to submit cows for first service. In this regard, the effect of extending the VWP from 60 to 88 DIM on first service P/AI and subsequent reproductive performance in the experiment described above may have been specific to the use of only TAI with a GnRH-based fertility protocol (i.e., Double-Ovsynch). For example, using all TAI results in a narrow range of DIM to first service regardless of the ability of cows to display estrus. This narrow range reduces variation of not only DIM to first service but also for second and greater AI services. By resolving anovulation, proper synchronization of ovulation, and optimization of the endocrine environment before insemination in a majority of cows (Souza et al., 2008; Herlihy et al., 2012; Giordano et al., 2013), GnRH-based protocols also may offset the detriment of shorter VWP on P/AI to a greater extent than programs not including synchronization of ovulation or synchronization of ovulation with PGF-based protocols. Thus, the method of submission to first service and the type of synchronization of ovulation protocol, if employed, are important considerations at the time of defining the duration of the VWP. In this regard, many dairy farms continue to submit cows for first service through a combination of detection of estrus and TAI after the Presynch-Ovsynch protocol. Cows detected in estrus after Presynch (two PGF treatments 14 days apart) are inseminated, whereas the rest of the cows receive TAI after completion of the protocol. In this case many farms use a VWP of approximately 50 to 60 DIM. Therefore, a reasonable question is how programs that combine AI at detected estrus and TAI compare with all TAI programs at different VWP durations.

Effect of a Combined Approach versus All TAI and Different VWP on Reproductive Performance of Dairy Cows

As part of the experiment described above in one of the participating farms we also included an additional treatment that consisted of a typical combined program with the Presynch-Ovsynch (PSOv) protocol. Cows in this treatment were allowed to be inseminated at detected estrus any time after 50 ± 3 DIM coincident with the second PGF treatment of Presynch. The other two treatments consisted of all TAI after the Double-Ovsynch protocol at 60 ± 3 (DO60) or 88 ± 3 (DO88) DIM as described. Cows in the three treatments were managed similarly for second and greater AI services.

Our most relevant findings were that cows managed for first service with the combined approach of AI after detected estrus and VWP of 50 DIM had similar time to pregnancy during lactation to cows managed with all TAI and VWP of 60 DIM. In addition, cows in both treatments with a shorter VWP became pregnant at a faster ($P < 0.05$) rate than cows in the DO88 treatment (Figure 2). As a result, median and mean days to pregnancy were 90 and 123 for DO60, 96 and 126 for PSOv, and 116 and 150 for DO88. The hazard of pregnancy was greater ($P < 0.01$) for cows in the DO60 than the DO88 treatment (HR 1.53, 95% CI: 1.32 to 1.78) and for cows in the PSOv than the DO88 treatment (HR 1.37, 95% CI: 1.19 to 1.61). No differences were observed between cows in the DO60 and PSOv treatments (HR 1.12, 95% CI: 0.96 to 1.30).

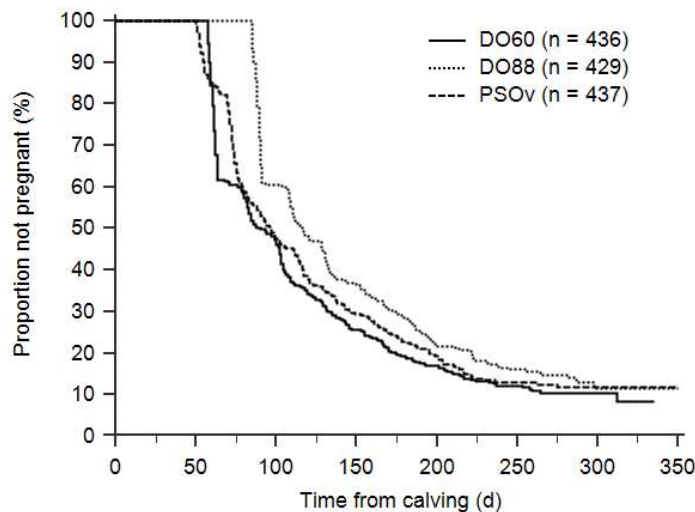


Figure 2. Survival curves for time to pregnancy after calving for cows that received first service through a combination of detection of estrus and TAI with the Presynch-Ovsynch protocol (PSOv) or all TAI at 60 (DO60) or 88 DIM (DO88) after the Double-Ovsynch protocol. Cows in the PSOv and DO60 treatments became pregnant faster ($P < 0.05$) than cows in the DO88 treatment.

Although the overall effect of VWP duration on P/AI at first service followed the same trends (no statistically significant differences were detected likely because of a lack of statistical power) as for the larger experiment (presented previously), the positive effect of longer VWP on this farm was not as dramatic favoring the groups with shorter VWP. This was particularly important for multiparous cows that had the same P/AI after all TAI at 60 or 88 DIM and approximately 4 percentage points fewer overall P/AI for cows in the Presynch-Ovsynch treatment.

Thus, we concluded that first-service management programs that result in a similar range of DIM to first service regardless of being a combined approach (e.g., AI at detected estrus and TAI with Presynch-Ovsynch with 50-day VWP) or all TAI with Double-Ovsynch and 60-day VWP) can lead to similar time to pregnancy after calving. In addition, these programs with shorter VWP and similar range of DIM at first

service can reduce time to pregnancy when compared with an all TAI program with an extended VWP (i.e., 88 DIM), which did not result in a substantial increment in first service P/AI. Our observations are particularly important for herds that extend the duration of the VWP and do not observe a substantial increment in first service P/AI. Indeed, in our study we estimated that to have the same proportion of pregnant cows at approximately 90 DIM, P/AI at first service for the program with extended VWP must be 10 to 11 percentage points greater for primiparous cows and 7 to 12 percentage points greater for multiparous cows.

Because economic differences between reproductive management programs depend on multiple factors beyond timing of pregnancy during lactation as clearly demonstrated in our comparison of all TAI at 60 vs 88 DIM, it remains to be determined which one of the strategies was the most profitable in our experiment for programs using a combination of AI at detected estrus and TAI vs. all TAI.

■ Conclusions

Manipulating the duration of the VWP affects herd reproductive performance, exit dynamics (i.e., cow sales), and profitability. From a reproductive performance perspective the greatest effect of delaying the end of the VWP is greater P/AI to first service (in particular for primiparous cows) and an overall delay in time to pregnancy, which may increase the risk of leaving the herd (in particular for multiparous cows). Extending the duration of the VWP as in our experiments may increase profitability of primiparous cows and reduce profitability of multiparous cows. Such effect would depend mostly on the herd replacement dynamics and milk production efficiency.

First-service management strategies that combine insemination of cows at detected estrus for first service with TAI in the absence of previous first AI at estrus may result in similar days to pregnancy during lactation provided that average DIM at first service is similar for all TAI programs and first service P/AI is reasonable. Management programs that reduce DIM at first service through AI at detected estrus and TAI or all TAI can reduce time to pregnancy compared with all TAI programs with longer VWP, in particular, when the extension of the VWP does not substantially increase first service P/AI.

Collectively, data from our recent research indicate that the effect of VWP duration and first service management strategies on dairy herd performance depends upon complex interactions between the pattern of insemination for first service, pregnancy per AI, and herd exit dynamics, all of which may vary for primiparous and multiparous cows. As a result, dairy managers should consider these complex interactions when defining VWP duration for their lactating dairy cows.

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Daily Profit of 3-Breed Crossbreds Compared to Holsteins – Our Experience from a 10-Year Designed Study

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■ Take Home Messages

- ▶ Unlike previous research on crossbreeding of dairy cattle, this 10-year study had controlled and balanced enrolment of foundation cows, was carefully designed, used high-ranking artificial insemination (AI) bulls for all breeds, and had uniform data recording in high-production herds.
- ▶ The use of the Holstein, Viking Red, and Montbéliarde breeds in a 3-breed rotational program is marketed as ProCROSS®.
- ▶ Daily fat + protein production for lifetimes of cows was 1% higher for 2-breed crossbreds (Viking Red×Holstein and Montbéliarde×Holstein) and 1% lower for 3-breed crossbreds compared with their Holstein herdmates.
- ▶ All generations of the crossbred cows had lower stillbirth rates, and the 3-breed crossbred calves born to 2-breed crossbred dams had one-half the number of stillborn calves at 1st calving compared with their Holstein herdmates.
- ▶ The 2-breed crossbreds had 12 fewer days open and the 3-breed crossbreds had 17 fewer days open compared with their Holstein herdmates.
- ▶ Health treatment cost was 23% lower for the 2-breed crossbreds and 17% lower for 3-breed crossbreds compared with their Holstein herdmates.
- ▶ Lifetime death loss was 4% lower for both the 2-breed crossbreds and the 3-breed crossbreds compared with their Holstein herdmates.
- ▶ The combined 2-breed and 3-breed crossbreds had 153 more days in the herd compared with their Holstein herdmates. Therefore, replacement cost was substantially lower for both the 2-breed and 3-breed crossbreds compared with their Holstein herdmates.
- ▶ Daily profit was 13% higher for the 2-breed crossbreds and 9% higher for the 3-breed crossbreds compared with that of their Holstein herdmates.
- ▶ The average inbreeding coefficient of U.S. Holstein females born in 2020 surpassed 8.5%, and the rate of annual increase in average inbreeding currently exceeds 0.4%, which seems to be an unsustainable annual increase into the future.
- ▶ Hybrid vigour from crossbreeding is most influential for traits related to fertility, health, and survival, and it falls on top of genetic improvement within breeds.

■ Motivation for the Study

Interest in crossbreeding of dairy cattle continues to increase globally. Selection in the Holstein (HO) breed for milk production has been tremendously successful over the past 40 years. That success resulted in the HO breed becoming almost a monoculture for milk production globally at the start of the 21st century. However, the HO breed has also selected strongly for larger body size and more angularity of cows on top of the selection for production. All three of these traits **have genetic antagonism with** fertility, health, and

survival of cows; therefore, the HO breed experienced a rapid decline in these functional traits. In recent years, selection emphasis for the HO breed has shifted more to these functional traits, but they have low genetic control and continue to be antagonistic with the continued selection for production.

The adoption and use of genomic selection in the U.S. over the past 12 years has substantially accelerated the annual increase of average inbreeding of HO females, mostly due to a dramatic reduction of generation interval (time between each generation). Therefore, the average inbreeding coefficient of U.S. Holsteins rose to 8.59% for females born during 2020. For reference, the inbreeding resulting from a bull mated to his own daughter averages 25% and the mating of first cousins results in inbreeding averaging 6.25%, which is the level of average inbreeding U.S. HO females surpassed in 2014. More concerning is the acceleration of average inbreeding in recent years because average inbreeding has increased about 0.40% in each of the past five years. Inbreeding depression silently steals profit from dairy producers because it is expressed mostly for traits that are not readily noticeable such as embryo loss, less disease resistance, and reduced survival.

Hybrid vigour from crossbreeding is expressed as an opposite effect of inbreeding depression. When parents of different breeds are mated to create a crossbred offspring, the two genes at the same location on the chromosomes cannot be identical from a common ancestor. Therefore, recessive genes of both major and minor consequence are not likely to be expressed with crossbreeding. Hybrid vigour has been embraced by the pig, beef, sheep, chicken, and turkey industries for more than 40 years.

Hybrid vigour does not replace genetic improvement within breeds, which increases the frequency of desirable genes. The support for, and the stewardship of, breeds with robust genetic improvement programs is critical for successful crossbreeding programs. Dairy producers should select three breeds of dairy cattle that are appropriate for their specific management systems and use the highest-ranking AI bulls from each of the three breeds.

This 10-year study compared cows from 3-breed rotational crossbreeding using the HO, Viking Red (VR), and Montbéliarde (MO) breeds with their pure HO herd mates. The study was initiated in 2008 and continued through 2017. This 3-breed crossbreeding program is referred to as ProCROSS[®] and is jointly marketed by two breeding companies (Viking Genetics and Coopex Montbéliarde). The VR breed is the result of combining the genetic improvement programs of the previously separate Swedish Red, Finnish Ayrshire, and Danish Red breeds.

■ Design of the Study

Seven Minnesota herds were enrolled in the study in 2008, and the managers of the herds committed 3,550 HO virgin heifers and cows as the 'foundation' females. The herds were elite for production. At the end of the study in December 2017, the seven herds had average production of 13,587 kg milk, 512 kg fat, and 426 kg protein with an average herd size of 982 cows. All herds fed cows a total mixed ration, and lactating cows were housed in free-stall confinement barns.

This study is unique, because no previous study on crossbreeding with U.S. commercial dairy herds was carefully designed regarding matings across generations. Each of the seven herds in the study offered a minimum of 250 foundation HO females, which were assigned by researchers to be mated so their descendants across generations would be either HO or ProCROSS. The foundation females were paired and assigned to the two breed types based on their age (for heifers), lactation number (for cows), sire, and production level.

At least 150 foundation females were mated in each herd to HO AI bulls as were their descendants across generations. Also, at least 100 foundation HO females were mated in each herd to either VR or MO AI bulls (in equal number) to initiate a 3-breed rotational program in both directions. The 2-breed crossbred offspring of the foundation females were mated to the third breed to create 3-breed crossbreds. Finally, all 3-breed

crossbreeds were mated to HO AI bulls to keep the rotation moving forward. The two alternative rotations of breeds in each direction continued in successive generations in a designated order such as in Figure 1.

Some of the herds decided to enroll more than 250 HO foundation females in the study, and those herds chose which breed type the additional foundation females would be assigned. The enrolment by individual herds ranged from 250 to 785 foundation females, and 44% of these were mated to HO AI bulls and 56% were mated equally to VR and MO AI bulls. Female progeny were housed and managed together in the herds and treated the same in all ways including age at first breeding, health treatment, and culling.

Semen from proven AI bulls from the three breeds was used to breed heifers and cows in the study. Producers chose the bulls in consultation with two genetic advisors of Minnesota Select Sires Co-op, Inc. Semen from the VR and MO bulls was imported to the U.S. by Creative Genetics of California and ranked highly for the Nordic Total Merit index or the French ISU index, which are the national indices for the 2 breeds. Herd managers were asked to select proven HO AI bulls ranking among the top 10% for the U.S. Net Merit Index, and all of the HO AI bulls were marketed by Select Sires, Inc.

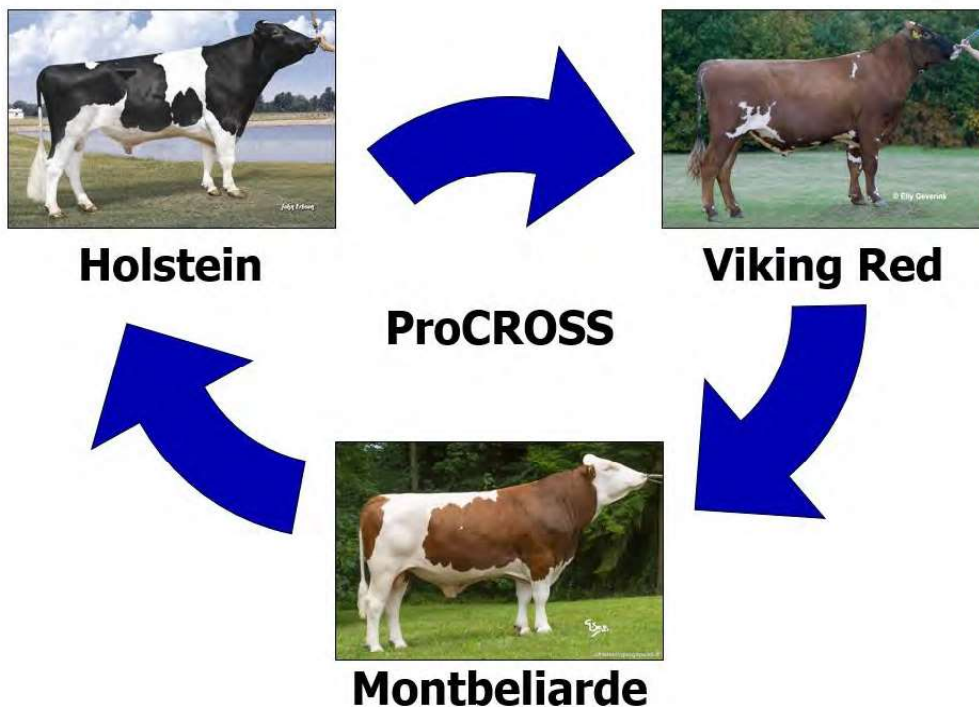


Figure 1. ProCROSS rotation initiated with Viking Red on Holstein female.

All heifers and cows were mated to individual AI bulls by the two genetic advisors with corrective mating for conformation. Furthermore, all matings of HO AI bulls with HO cows received inbreeding protection. The 2-breed crossbreeds and their HO herdmates calved a first time starting in December 2010. The 3-breed crossbreeds and their HO herdmates began calving in November 2012.

The window of time for each generation of cows in the study wasn't distinct and the generations overlapped, especially in the later years of the study. For example, some HO cows were herdmates with 2-breed as well as some 3-breed crossbreeds. Observations were recorded until data collection ended on December 31, 2017. The experimental design, methods of analysis, and results of the study are provided in the scientific literature (Hazel, et al., 2020a,b; Hazel, et al., 2021).

■ Analysis

Traits were analyzed within lactation number and also separately for the 2-breed crossbred and 3-breed generations and their HO herdmates. Cows that started lactation with an abortion (gestation length less than 260 days) were removed from the analysis of gestation length, stillbirth, fertility, production, and conformation, but they were included for the analysis of health treatment cost and survival. Also, cows sold for dairy purposes were excluded from the analysis of survival. Three seasons of calving were defined as January to April, May to August, and September to December for each herd. Cows that calved during seasons with fewer than three crossbred and three HO cows of the same generation and lactation number were removed from the analysis for a trait. Therefore, the number of cows analyzed varied somewhat from trait to trait. All traits were analyzed accounting for the effects of lactation number, herd-season of calving, and breed type of cow (such as VR×HO, MO×HO, and HO for the 1st generation).

Results are provided with probabilities that indicate whether reported differences are large enough to be statistically significant (can be taken seriously). The probability of $P < 0.10$ (symbolized with “+”) indicates a difference with a 90% certainty of being real rather than due to chance. The probability of $P < 0.05$ (symbolized with “*”) indicates a difference with a 95% certainty, and $P < 0.01$ (symbolized with “**”) indicates a difference with a 99% certainty of being real rather than due to chance. The lack of a symbol indicates a difference may simply be due to chance.

■ Results

Stillbirth

Stillbirth rates were numerically lower for all of the crossbred types at first calving and were significantly lower for both types of 2-breed crossbreds and about one-half lower for the VR×MO/HO 3-breed crossbreds compared with their HO herdmates (Table 1). Stillbirth rates were lower for cows at second and third calving regardless of breed type, and the differences were not statistically significant for the breed types.

Fertility

Both the VR×HO and MO×HO 2-breed crossbreds had significant advantages for fertility compared with their HO herdmates (Table 1). Averaged across lactations, the 2-breed crossbreds had 7.3% higher conception rate at first breeding compared with their HO herdmates, and the 2-breed crossbreds also had fewer times bred (−0.2 to −0.4) compared with their HO herdmates in first and second lactation. Days open averaged 134 days across the lactations for the HO herdmates, which is superior to the average of 145 days open of herds enrolled in U.S. dairy records management systems. However, the VR×HO 2-breed crossbreds averaged 8 fewer days open and the MO×HO 2-breed crossbreds averaged 17 fewer days open compared with their HO herdmates across lactations. Pregnancy rate was four percentage points higher for the 2-breed crossbreds compared with their HO herdmates across lactations.

Table 1. Fertility of VR×HO and MO×HO 2-breed crossbreds compared with their HO herdmates.

	Holstein		Difference from Holstein			
	Cows (n)	Average	Cows (n)	Difference	Cows (n)	Difference
First lactation						
Conception rate at first breeding	1,125	37%	558	+8%**	534	+6%*
Overall conception rate	1,127	37%	558	+4%*	534	+8%**
Times bred (up to five)	1,142	2.4	564	-.2*	541	-.3**
Days open	1,061	127	541	-7	517	-12**
Pregnancy rate	1,061	27%	541	+3%	517	+5%**
Second lactation						
Conception rate at first breeding	822	29%	425	+7%**	409	+11%**
Overall conception rate	824	31%	427	+4%*	410	+9%**
Times bred (up to five)	840	2.6	435	-.3**	424	-.4**
Days open	704	139	382	-11*	387	-22**
Pregnancy rate	704	24%	382	+3%*	387	+7%**
Third lactation						
Conception rate at first breeding	389	30%	251	+1%	253	+11%**
Overall conception rate	391	31%	252	+1%	255	+9%**
Times bred (up to five)	411	2.5	259	0	266	-.3*
Days open	336	143	231	-4	245	-23**
Pregnancy rate	336	23%	231	+1%	245	+7%**

* $P \leq 0.05$, ** $P \leq 0.01$ significantly different from Holstein.

The 3-breed crossbreds also were also superior for fertility compared with their HO herdmates for all fertility traits (Table 2). The HO herdmates had reasonable conception rate at first breeding (43% in first lactation and 35% for second and third lactation). Yet, the conception rate at first breeding was 8.7% higher for the 3-breed crossbreds averaged across lactations compared with their HO herdmates. Days open were more than two weeks fewer in first lactation, 2.5 weeks fewer in second lactation, and almost three weeks fewer in third lactation for the 3-breed crossbreds compared with their HO herdmates. The pregnancy rate superiority of the 3-breed crossbreds compared with their HO herdmates was +5% to +11%.

Concern about fertility is often the primary reason dairy producers consider crossbreeding. The superior fertility of the crossbreds may have mostly resulted from hybrid vigour. However, the outstanding performance of the 3-breed crossbreds compared with their HO herdmates is likely also because of the direct contributions of the VR and MO breeds, which have selected heavily for improved fertility for many years. Perhaps, more importantly, the VR and MO breeds haven't selected against body condition of cows. The relationship between less body condition and poor fertility of dairy cows is well established.

Table 2. Fertility of VR×MO/HO and MO×VR/HO 3-breed crossbreeds compared with their HO herdmates.

	Holstein		Difference from Holstein			
	Cows (n)	Average	Cows (n)	Difference	Cows (n)	Difference
First lactation						
Conception rate at first breeding	1,124	43%	515	+9%**	458	+8%**
Overall conception rate	1,042	41%	491	+7%**	446	+9%**
Times bred (up to five)	1,033	2.2	491	-.3**	455	-.2**
Days open	1,022	126	471	-15**	433	-16**
Pregnancy rate	1,022	28%	471	+6%**	433	+7%**
Second lactation						
Conception rate at first breeding	612	35%	331	+7%†	292	+12%**
Overall conception rate	535	36%	301	+6%*	269	+8%**
Times bred (up to five)	566	2.4	306	-.2*	282	-.3**
Days open	512	134	283	-20**	263	-17**
Pregnancy rate	512	25%	283	+8%**	263	+6%**
Third lactation						
Conception rate at first breeding	250	35%	164	+7%	153	+13%*
Overall conception rate	215	33%	141	+11%**	132	+15%**
Times bred (up to five)	226	2.4	144	-.5**	142	-.5**
Days open	183	134	128	-15†	124	-25**
Pregnancy rate	183	25%	128	+5%†	124	+11%**

† $P \leq 0.10$, * $P \leq 0.05$, ** $P \leq 0.01$ significantly different from Holstein.

Health Treatment Cost

Health treatment cost of dairy cows is not well-documented in the U.S. because of the lack of complete and uniform recording. The seven herds in the study consistently recorded 16 types of health treatment events for the duration of the study. The veterinarians who provided service to the herds were interviewed, and they shared estimates of cost for medication and cost for veterinary care of specific types of treatment. Furthermore, the herd managers provided the estimated time required by their staff to restrain cows and to administer treatment. The time required for a treatment was converted to a labour cost for each of the 16 treatment types. The health treatment costs of a cow were assigned to each treatment event and added across lactation. This allowed multiple treatments of the same type for a cow within lactation. The cost of the 16 types of health treatment cost was added within each lactation of a cow, and this approach permitted a robust distribution of total health treatment costs for cows.

Health treatment cost for first lactation cows was low compared with the cost for second and third lactation cows for all breed types of cows (Table 3). The MO×HO 2-breed crossbreeds had significantly lower total health treatment cost (-28%) compared with their HO herdmates during first lactation. The VR×HO 2-breed crossbreeds had a tendency for lower total health treatment cost (-16%) compared with their HO herdmates during first lactation. Total health treatment cost during first lactation wasn't significantly different for the 3-breed crossbreeds compared with their HO herdmates. In general, the seven herds had relatively low health treatment cost for all breed types during the first lactations of cows. However, differences between the breed types were noticeable in second and third lactation. Overall, the 2-breed crossbreeds averaged 23% less total health treatment cost and the 3-breed crossbreeds averaged 17% less total health treatment cost across their first three lactations compared with their pure HO herdmates.

Table 3. Total health treatment cost for 2-breed and 3-breed crossbreds compared with their HO herdmates.

Lactation number	Holstein		Difference from Holstein			
	Cows (n)	Average	Cows (n)	VR×HO Difference	Cows (n)	MO×HO Difference
First	1,280	\$43	624	-\$7†	592	-\$12**
Second	1,007	\$68	498	-\$20**	471	-\$21**
Third	577	\$92	328	-\$14**	334	-\$25**

Lactation number	Holstein		Difference from Holstein			
	Cows (n)	Average	Cows (n)	VR×MO/HO Difference	Cows (n)	MO×VR/HO Difference
First	1,186	\$43	537	-\$3	502	-\$5
Second	654	\$81	333	-\$15**	305	-\$27**
Third	267	\$109	158	-\$18*	147	-\$37**

† P ≤ 0.10, * P ≤ 0.05, ** P ≤ 0.01 significantly different from Holstein.

Health treatment cost was broken down into five categories:

- mastitis (including mastitis diagnostic tests)
- lameness
- reproductive (retained placenta, metritis, cystic ovary, and other reproductive)
- metabolic (milk fever, displaced abomasum, ketosis, and digestive)
- miscellaneous (respiratory, injury, and all other).

Analysis of each health category indicated differences in total health treatment cost came mostly from three of the five categories — less mastitis, less metabolic, and less miscellaneous treatment cost — for both the 2-breed and 3-breed crossbreds compared with their HO herdmates.

Health treatment cost reported in this study was the economic cost for treatment of disease, but that cost may be a conservative reflection of the actual difference in disease status of the crossbreds compared with their HO herdmates. Recording of treatment events excluded subclinical disease that may have gone undetected, time for disease recovery, and time spent in a hospital pen. The gains from hybrid vigour for these sorts of health events, which are difficult to accurately record, were not captured. Furthermore, culling records from the herds revealed they often chose to cull or euthanize cows with diseases that had high treatment cost and cows with poor recovery prognosis.

Dairy breeding companies are now emphasizing health traits for genetic improvement of HO cows. However, slow progress is expected within a breed, because the genetic control of health is much less (heritability of 1% to 3%) than the genetic control of other traits such as production and conformation. The exploitation of hybrid vigour for improved cow health in addition to genetic improvement within breeds is expected to be more effective in achieving improved cow health than relying only on genetic improvement within a breed.

Survival

Despite the low average health treatment cost of first lactation HO cows in this study, significantly more of the VR×HO 2-breed and MO×HO 2-breed crossbreds calved a second time within 14 months and 17 months than did their HO herdmates (Table 4). The obvious explanation is the superiority of the 2-breed

crossbreeds for fertility compared with their HO herdmates in first lactation (Table 1). More VR×HO and MO×HO 2-breed crossbreeds calved a third time within 14 months (+8% and +16%) and within 17 months after second calving (+6% and +12%) compared with their HO herdmates (Table 4).

Table 4. Survival of VR×HO and MO×HO 2-breed crossbreeds compared with their HO herdmates.

	Holstein		Difference from Holstein			
	Cows (n)	Average	VR×HO		MO×HO	
			Cows (n)	Difference	Cows (n)	Difference
Virgin heifers						
Survival to first calving	1,581	86.7%	706	+3.0%†	695	+2%
First lactation						
Second calving within 14 months	1,250	63%	608	+6%*	582	+8%**
Second calving within 17 months	1,239	76%	604	+5%*	576	+6%**
Death loss	1,223	4%	593	-1%	568	-1%
Second lactation						
Third calving within 14 months	983	46%	496	+8%**	470	+16%**
Third calving within 17 months	980	61%	496	+6%†	470	+12%**
Death loss	959	8%	483	-4%**	451	-2%
Third lactation						
Fourth calving within 14 months	559	40%	322	+9%*	329	+19%**
Fourth calving within 17 months	549	51%	322	+6%	328	+18%**
Death loss	560	8%	319	0%	330	-3%
Survival to subsequent calving						
Survival to second calving	1,223	81%	593	+4%†	568	+4%†
Survival to third calving	1,201	51%	581	+7%*	551	+12%**
Survival to fourth calving	1,012	27%	550	+5%†	516	+16%**
Died up to 45 months (after first calving)						
	640	16.3%	376	-5.1%**	358	-2.6%
Lived to at least 45 months (after first calving)						
	640	18.0%	376	+6.7%**	358	+15.2%**

† P ≤ 0.10, * P ≤ 0.05, ** P ≤ 0.01 significantly different from Holstein.

The percentage of cows that calved again within 14 and 17 months measured both the time required for cows to calve again as well as whether they actually calved again. Few cows required longer than 17 months to calve again, because the herds in the study culled aggressively for both fertility and persistency of production in later lactation.

Actual survival to next calving is also in Table 4, and cows were required to calve a first time in order to be included. For the 2-breed crossbreeds, 58% of the VR×HO and 63% of the MO×HO calved a third time, but only 51% of their HO herdmates calved a third time. Survival to fourth calving was even more different for the 2-breed crossbreeds compared with their HO herdmates, because 32% of the VR×HO, 43% of the MO×HO, and 27% of the HO calved a fourth time.

Death loss within lactation was the number of cows that died divided by the number of cows that calved in that lactation (Table 4). Death loss was low during first lactation for all breed types. However, during second lactation, the VR×HO 2-breed crossbreeds had 4% death loss compared with 8% death loss of their HO herdmates. Death loss was also compared up to 45 months after first calving. The HO herdmates of the

crossbreds had 16.3% death loss, which is similar to the 15% to 16% death loss of HO cows born in 2008 to 2012 that are used for U.S. genetic evaluation. However, the VR×HO 2-breed crossbreds had significantly less death loss (11.2%) compared with their HO herdmates.

Table 5. Survival for VR×MO/HO and MO×VR/HO 3-breed crossbreds compared with their HO herdmates.

	Holstein		Difference from Holstein			
			VR×MO/HO		MO×VR/HO	
	Cows (n)	Average	Cows (n)	Difference	Cows (n)	Difference
Virgin heifers						
Survival to 1st calving	1,557	85.7%	667	+3.9%*	613	+3.1%
First lactation						
Second calving within 14 months	1,103	63%	506	+9%**	474	+6%†
Second calving within 17 months	1,018	76%	475	+5%	447	+1%
Death loss	1,057	4%	490	-2%	456	+1%
Second lactation						
Third calving within 14 months	586	48%	297	+16%**	275	+19%**
Third calving within 17 months	545	61%	273	+14%**	252	+13%**
Death loss	569	7%	293	-4%**	269	-3%
Third lactation						
Fourth calving within 14 months	202	37%	129	+29%**	115	+23%**
Fourth calving within 17 months	165	44%	97	+25%**	97	+21%**
Death loss	181	6%	111	-1%	103	-3%
Survival to subsequent calving						
Survival to second calving	1,057	84%	490	+4%*	456	-1%
Survival to third calving	681	51%	318	+14%**	309	+8%*
Survival to fourth calving	311	22%	124	+24%**	135	+15%**
Died up to 45 months (after first calving)						
	250	12.4%	109	-6.0%†	117	-3.0%
Lived to at least 45 months (after first calving)						
	250	17.6%	109	+13.6%**	117	+8.9%*

† $P \leq 0.10$, * $P \leq 0.05$, ** $P \leq 0.01$ significantly different from Holstein.

The VR×MO/HO 3-breed crossbreds (89.6%) and the MO×VR/HO 3-breed crossbreds (88.8%) had significantly higher survival rates compared with their HO herdmates (85.7%) from two days of age to first calving (Table 5). More of the 3-breed crossbreds (+6% and +9%) had a second calving within 14 months compared with the 63% survival rate of their HO herdmates (Table 5). For cows that calved a second time, significantly more 3-breed crossbreds calved a third time, and the difference for survival rate was 16% to 19% higher by 14 months after calving and 13% to 14% by 17 months after first calving compared to that of their HO herdmates. Differences between the breed types grew even larger for cows that calved a fourth time within 14 months of third calving because the VR×MO/HO (66%) and MO×VR/HO (60%) 3-breed crossbreds had a significantly higher survival rate compared with their HO herdmates (37%).

For the 3-breed crossbreds, death loss within each of the lactations was about one-half that of their HO herdmates. The 3% death loss of the VR×MO/HO crossbreds in second lactation was significantly lower than the 7% death loss of their HO herdmates. Up to 45 months after first calving, 6.4% of the VR×MO/HO and 9.4% of the MO×VR/HO 3-breed crossbreds died, but 12.4% of their HO herdmates died (Table 5).

The percentage of cows that survived to at least 45 months after first calving was 29% for the 2-breed crossbreds and 3-breed ProCROSS combined. On the other hand, only 18% of their HO herdmates survived to at least 45 months after first calving. Replacement cost is among the top three expenses for most herds.

Production

Actual (not mature equivalent) 305-day production was estimated from test-day records of milk, fat, and protein. Cows were required to have at least two test days to be included in the analysis. Cows that were milked longer than 305 days in lactation were limited to 305 days of production. Cows that became pregnant quickly after calving, which resulted in lactations less than 305 days, and cows that left the herd prior to 305 days in milk had their lactations projected to 305 days.

Lifetime production of a cow was actual daily production added across all days she was in the herd. Production from lactations longer than 305 days were included, and lactations of cows that were less than 305 days were not projected to 305 days for lifetime production. Cows that did not survive to a first test day were assigned 11.3 kg of milk, 0.397 kg of fat, and 0.340 kg of protein production for each day between calving and removal from the herd.

For lifetime production, 315 cows (23%) of the first-generation cows lived beyond 45 months in the herd or were still in the herd at the end of the study. For second generation cows, 100 cows (21%) lived beyond 45 months. For these cows, additional daily production was projected by multiplying the production per day up to 45 months after first calving by the predicted number of additional days that the cow remained in the herd. The projected production of each cow after 45 months was added to the production during their initial 45 months in the herd. Daily production for each cow was her lifetime production divided by the number of days she was in the herd (or her projected days in the herd) including the dry period. All the herds routinely milked most of their cows three times daily.

The herds had a young average age at first calving compared with the U.S. average. Age at calving was not significantly different for the 2-breed crossbreds compared with their HO herdmates for both first and second lactation (Table 6), and the MO×HO 2-breed crossbreds had a tendency to calve one-half month earlier compared with their HO herdmates in third lactation.

The fat + protein production of the MO×HO 2-breed crossbreds was 3% higher compared with that of their HO herdmates in first and second lactation. Furthermore, the VR×HO 2-breed crossbreds were not significantly different from their HO herdmates for fat + protein production in any of the lactations. For fluid milk volume, the VR×HO 2-breed crossbreds were significantly lower compared with their HO herdmates, but the MO×HO 2-breed crossbreds were not significantly different from their HO herdmates. The majority of dairy herds in the world are paid for the fat and protein solids in milk rather than the volume of fluid that carries the solids. Cows with extra fluid carrier (water) are often considered to be less desirable because more expense is needed to cool and ship the additional fluid carrier. Therefore, most dairy producers believe less fluid volume with higher percentages of solids is advantageous.

On a lifetime basis, both the VR×HO 2-breed crossbreds (+96 days) and the MO×HO 2-breed crossbreds (+219 days) had significantly more longevity (days) in the herd compared with their HO herdmates. The differences for days are equivalent to +3.2 months for the VR×HO 2-breed crossbreds and +7.2 months for the MO×HO 2-breed crossbreds compared with their HO herdmates. The additional length of time in the herd resulted in significantly more lifetime fat + protein production for the MO×HO 2-breed crossbreds compared with their HO herdmates (Table 6). For daily fat + protein production across the lifetimes of cows, the VR×HO 2-breed crossbreds had 1% less and the MO×HO 2-breed crossbreds had 2% more daily fat + protein production compared with their HO herdmates.

The 3-breed crossbreds had significantly younger age at calving for first, second, and third lactation compared with their HO herdmates (Table 7). On average, the 3-breed crossbreds calved 12 days sooner

for first lactation, 21 days sooner for second lactation, and 49 days sooner for third lactation compared with their HO herdmates. The explanation for their younger ages at calving was their advantage for fertility compared with their HO herdmates (Table 2).

The VR×MO/HO and MO×VR/HO 3-breed crossbreds had significantly lower fat + protein solids production during first (−4%), second (−3%), and third (−4%) lactation compared with their HO herdmates (Table 7). The lower 305-day production was not surprising for two reasons: 1) the 305-day production wasn't adjusted for the fewer days open (their advantage for fertility) of the 3-breed crossbreds compared with their HO herdmates, and fewer days open lowers production of cows in the final trimester of pregnancy, and 2) the 3-breed crossbreds had an average HO content of only 25%.

Table 6. Production (actual, not mature equivalent) for 305-day lactations, for lifetime, and per day for the 2-breed crossbreds compared with their HO herdmates.

	Holstein	Difference from Holstein	
		VR×HO	MO×HO
First lactation			
Cows (number)	1,180	582	556
Age at calving (months)	23.8	0	0
305-day fat + protein (kg)	765	+11	+23**
305-day milk (kg)	11,378	−419**	+67
Second lactation			
Cows (number)	883	461	443
Age at calving (months)	36.5	−.1	−.1
305-day fat + protein (kg)	887	−5	+19**
305-day milk (kg)	13,338	−790**	−3
Third lactation			
Cows (number)	451	281	297
Age at calving (months)	48.9	−.4	−.5†
305-day fat + protein (kg)	927	0	+13
305-day milk (kg)	13,932	−665**	−72
Lifetime			
Cows (number)	640	376	358
Days in the herd	886	+96*	+219**
Fat + protein production (kg)	2,201	+196	+609**
Daily across lifetime			
Cows (number)	640	376	358
Fat + protein production (kg)	2.51	−.02**	+ .06**

† $P \leq 0.10$, * $P \leq 0.05$, ** $P \leq 0.01$ significantly different from Holstein.

On a lifetime basis, both the VR×MO/HO (+5.8 months) and MO×VR/HO (+3.8 months) 3-breed crossbreds had numerically more longevity compared with their HO herdmates, although the advantage of the MO×VR/HO crossbreds was not statistically significant. Because of their longer lives, both types of 3-breed crossbreds had numerically higher lifetime fat + protein production. For daily production of fat + protein production across their lifetimes, the VR×MO/HO 3-breed crossbreds were 2% lower and the MO×VR/HO 3-breed crossbreds were 1% higher compared with their HO herdmates.

ProCROSS© is a long-term and continuous rotational breeding program, and dairy producers must focus on the combined impact across generations of the rotation. When the daily fat + protein production of the VR×HO and MO×HO 2-breed crossbreds (−1% and +2%, respectively) and of the VR×MO/HO and

MO×VR/HO 3-breed crossbreds (−2% and +1%, respectively) are combined, the difference for daily fat + protein production from their HO herdmates was zero.

The daily fat + protein production of cows across their lifetimes is a more appropriate measure than 305-day production for comparing the differences between breed types. Daily fat + protein production includes the days that cows are dry. On average, the crossbreds in this study had more dry days compared with their HO herdmates because they calved more times during their lifetimes. However, calving more frequently also resulted in the crossbreds having more days during their lifetimes with peak production compared with their HO herdmates. If only lactating days of cows had been analyzed, the daily fat + protein production of the crossbreds would have been higher compared with their HO herdmates.

Table 7. Production (actual, not mature equivalent) for 305-day lactations, for lifetime, and per day for the 3-breed crossbreds compared with their HO herdmates.

	Holstein	Difference from Holstein	
		VR×MO/HO	MO×VR/HO
First lactation			
Cows (number)	1,073	505	462
Age at calving (months)	23.2	−.5**	−.3*
305-day fat + protein (kg)	795	−38**	−22**
305-day milk (kg)	11,803	−1,202**	−932**
Second lactation			
Cows (number)	582	309	291
Age at calving (months)	35.9	−.9**	−.6**
305-day fat + protein (kg)	906	−44**	−16†
305-day milk (kg)	13,551	−1,326**	−850**
Third lactation			
Cows (number)	228	143	134
Age at calving (months)	48.5	−1.7**	−1.6**
305-day fat + protein (kg)	953	−56**	−27*
305-day milk (kg)	14,295	−1,466**	−1,087**
Lifetime			
Cows (number)	250	109	117
Days in the herd	850	+176*	+117
Fat + protein production (kg)	2,132	+385†	+307
Daily across lifetime			
Cows (number)	250	109	117
Fat + protein production (kg)	2.55	−.06**	+.03**

† P ≤ 0.10, * P ≤ 0.05, ** P ≤ 0.01

Conformation and Body Condition Score

Conformation had a scale of one to nine, and cows were evaluated once each lactation between two and 150 days after calving (average of 35 days) by the two genetic advisors of Minnesota Select Sires Co-op, Inc. Cows were evaluated every lactation, and this approach is unique for cows as they aged, because cows aren't routinely evaluated for conformation after first lactation by U.S. breed associations.

Stature

Stature had a scale of 1 = shorter to 9 = taller. The HO herdmatres to the crossbreds increased in stature during first lactation (from 5.4 to 5.7). This result confirmed the HO herdmatres of crossbred cows in this study became taller with time, despite an effort by dairy producers to select for shorter stature of their HO cows. Both the VR×HO 2-breed (4.0) and MO×HO 2-breed (4.7) crossbreds were significantly shorter for stature compared with their HO herdmatres (5.4) in first lactation. This trend continued for second and third lactations. The 3-breed crossbreds were also shorter in stature compared with their HO herdmatres for all lactations. Furthermore, the VR×MO/HO 3-breed (4.3) and MO×VR/HO 3-breed (4.5) crossbreds were both intermediate for stature between the extremes of their 2-breed dams.

Angularity and Body Condition Score (BCS)

The scale for angularity was 1 = round to 9 = angular. Body condition score had a scale from 1 = thin to 5 = obese. As expected, angularity and BCS had a strong relationship. All breed types of cows became more angular (less BCS) with age. Also, both generations of crossbreds had less angularity and more BCS compared with their HO herdmatres. The MO×HO 2-breed (2.6) and MO×VR/HO 3-breed (2.6) crossbreds had the least angularity and MO×VR/HO 3-breed crossbreds had the highest BCS (3.90) for all breed types in first lactation. The relationship of lower BCS and reduced fertility, health, and survival of dairy cows is well documented.

Body Depth

Body depth had a scale of 1 = shallow to 9 = deep. All the 2-breed and 3-breed crossbreds had significantly shallower body depth than their HO herdmatres. Cows with more body depth are more prone to displaced abomasum after calving because the digestive tract has more space to move after the calf is born. Therefore, less body depth of the crossbreds compared with their HO herdmatres may have contributed to the lower incidence of metabolic treatment cost of the crossbreds compared with their HO herdmatres in this study.

Foot Angle

The scale for foot angle was 1 = low to 9 = steep. The VR×HO 2-breed crossbreds had significantly lower foot angle compared with their HO herdmatres, but only in second lactation (5.1 versus 5.6) and third lactation (4.9 versus 5.3). However, the MO×HO 2-breed crossbreds had significantly steeper foot angle compared with their HO herdmatres in all lactations. Likewise, the MO×VR/HO 3-breed crossbreds had steeper foot angle compared with their HO herdmatres. The VR×MO/HO 3-breed crossbreds had 0.5 steeper foot angle compared with their HO herdmatres during first lactation, probably due to an average MO content of 25%.

Udder Clearance

Udder clearance had a scale of 1 = low to 9 = high and was evaluated as distance of the udder floor from the hock and not as distance from the ground. Therefore, cows with shorter stature (and shorter legs) had a disadvantage for udder clearance compared with their HO herdmatres that had longer legs on average. In other words, udders with identical dimension received a lower score for cows with shorter stature. All the 2-breed and 3-breed crossbreds had less udder clearance compared with their HO herdmatres.

Rear Teat Width

The scale for rear teat width was 1 = wide to 9 = close. In first lactation, the 2-breed (5.6) and 3-breed (5.4) crossbreds averaged more width between the rear teats and had scores closer to the midpoint of 5 compared with their HO herdmatres (6.6). Also, the rear teat width became closer across the years of the

study for their HO herdmates in first lactation (6.5 to 6.7 to 6.8). In second and third lactation, the 2-breed and 3-breed crossbreds also had more width between the rear teats compared with their HO herdmates.

Dairy producers express frustration with rear teats of cows that are close (touch or cross), especially for robotic milking. Significantly fewer 2-breed crossbreds in this study had touching or crossing rear teats compared with their HO herdmates in first lactation (5% versus 13%), second lactation (17% versus 28%), and third lactation (17% versus 29%).

Teat Length

Teat length had a scale of 1 = short to 9 = long. The VR×HO 2-breed crossbreds were not different for teat length from their HO herdmates. However, the MO×HO 2-breed crossbreds had significantly longer teats compared with their HO herdmates in second lactation (+0.7) and third lactation (+0.4). The 3-breed MO×VR/HO crossbreds had slightly longer (+0.4 and +0.5) teat length on average compared with their HO herdmates in first and second lactation. Extremely short teats are a functional problem for cows.

Lifetime Profit and Daily Profit

To be included in the analysis of lifetime profit and daily profit, cows were required to have the opportunity (based on the cut-off of data at the end of the study) to survive to 45 months in the herd. Also, at least 20 cows were required for each breed type of either 2-breed crossbreds or 3-breed crossbreds and their HO herdmates. This requirement permitted a fair comparison of breed types for lifetime performance by eliminating comparisons that included a small number of cows within herd. Therefore, cows from only three of the seven herds were compared for lifetime performance.

Lifetime profit was estimated from the income and expense accumulated by each cow on a daily basis and added across all days in the herd after first calving (Table 8). The income from production came from daily production of milk, fat, protein, and other solids, as well as income or loss from SCS, with the cost of hauling and milk marketing subtracted for each cow. The production prices were from the U.S. Federal Milk Marketing Order for the Upper Midwest for 2013 to 2017. Average component prices used were US\$4.9650 per kg of fat, US\$5.8631 per kg of protein, and US\$0.6177 per kg of other solids.

Table 8. Income and expense to determine lifetime profit

	Value	Unit	Reference
Income (US\$)			
Milk price	\$38.01	100 kg	USDA FMMA 30 ¹
Live female calf	\$200	Calf	Lifetime Net Merit
Live male calf			
Holstein	\$100	Calf	Heins et al. (2012)
Crossbred	\$130	Calf	Study herds
Cull value during first lactation			
Holstein	\$876	Cow	Study herds
VR-sired crossbred	\$876	Cow	Study herds
MO-sired crossbred	\$1,033	Cow	Study herds
Cull value during second and later lactation			
Holstein	\$941	Cow	Study herds
VR-sired crossbred	\$1,049	Cow	Study herds
MO-sired crossbred	\$1,047	Cow	Study herds
Expense (US\$)			
Feed (during lactation)	\$0.2341	kg of DM	FINBIN ²
Lactation overhead	\$4.76	Day	FINBIN ²
Replacement ³	\$1,910	Cow	Tranel (2019)
Dry cow overhead (including feed)	\$3.50	Day	FINBIN ²
Breeding	\$27	Event	Study herds
Fertility hormones	\$18	Event	Study herds
Palpation	\$7	Event	Study herds
Hoof trimming	\$15	Event	Study herds
Carcass disposal	\$34	Cow	Study herds

¹ Upper Midwest average for 2013 to 2017

² Average for 2013-2017, Center for Farm Financial Management, University of Minnesota

³ Replacement expense varied based on age at first calving for each cow

Lifetime profit was projected for all cows that lived beyond 45 months in the herd by multiplying each cow's daily profit up to 45 months by the predicted number of additional days the cow remained in the herd based on the survival rates of cows in each herd. However, cows that were projected beyond 45 months did not receive additional income from cull value and were not assessed additional expense for replacement or carcass disposal.

Daily profit of a cow was lifetime profit divided by the number of days in the herd. The most appropriate measure of profitability of cows on an ongoing basis within a herd is the daily profit per unit of available stall, cubicle, or pasture space. The daily income from production was 1% lower for VR×HO 2-breed crossbreds and 2% higher for MO×HO 2-breed crossbreds compared with their HO herdmates (Table 9), and this difference agreed well with the difference of the 2-breed crossbreds compared with their HO herdmates for daily fat + protein production. The calf value averaged US\$0.07 more per cow per day for the 2-breed crossbreds compared with their HO herdmates (Table 9). The 2-breed crossbred cows had a 17% greater daily calf value compared with their HO herdmates because 1) crossbred male calves, particularly those with white faces from the MO breed, had a +US\$30 higher sale price than HO calves, 2) crossbred cows had fewer stillborn calves than their HO herdmates, and 3) the crossbred cows calved more times during their lifetimes compared with their HO herdmates.

Table 9. Daily income and expense that contributed to daily profit for VR×HO and MO×HO 2-breed crossbreds compared with their HO herdmates.

	Holstein	Difference from Holstein	
		VR×HO	MO×HO
Cows (number)	640	376	358
Income (US\$)			
Production	\$14.82	−\$.20**	+\$\$.31**
Calf value	\$.42	+\$\$.06**	+\$\$.07**
Cull value	\$.69	+\$\$.04**	−\$.03**
Total income (US\$)	\$15.92	−\$.09**	+\$\$.36**
Percentage difference from Holstein		−1%	+2%
Expense (US\$)			
Feed (during lactation)	\$5.33	−\$.11**	+\$\$.02**
Lactation overhead	\$4.19	−\$.05**	−\$.03**
Replacement	\$1.59	−\$.13**	−\$.27**
Dry cow overhead (including feed)	\$.42	+\$\$.03**	+\$\$.02**
Health treatment	\$.24	−\$.08**	−\$.06**
Breeding	\$.19	−\$.01**	−\$.02**
Total expense (US\$)	\$12.19	−\$.33**	−\$.36**
Percentage difference from Holstein		−3%	−3%

** P ≤ 0.01 significantly different from Holstein.

The average cull value for VR×HO 2-breed crossbreds (US\$908) and MO×HO 2-breed crossbreds (US\$906) was significantly higher compared with that of their HO herdmates (US\$785). However, these differences were not obvious when the cull value was divided by days in the herd to obtain daily cull value (Table 9), because the crossbreds averaged 158 more days in the herd compared with their HO herdmates. The total of production income, calf value, and cull value resulted in a daily total income of US\$16.05 for the combined 2-breed crossbreds, which was 1% higher compared with US\$15.92 for their HO herdmates.

Expense for feed while cows were lactating was the single largest expense for cows in the study. Individual feed intake was not available for cows. Therefore, feed intake was predicted from the formulas of the National Research Council. Feed intake was independently assigned for each cow on each day and depended on the week of lactation, on daily fat-corrected milk, and on body weight of cows. Body weight was not available for cows in the study, so body weight was set to 567 kg for all first lactation cows and 680 kg for all second and later lactation cows regardless of breed type to estimate daily feed intake. Feed expense was calculated by multiplying estimated dry matter intake by US\$0.2341, which was the fixed cost of a kg of dry matter.

Replacement cost was variable for cows and differed based on age at first calving. The average age of first calving across breed types and years in this study was 23.4 months, and heifers calving the first time at 23.4 months had a replacement cost of US\$1910. Heifers calving at younger or older ages had US\$2.40 per day deducted or added to the replacement cost of US\$1910. For lifetime replacement cost, the 2-breed crossbreds (US\$1927) and their HO herdmates (US\$1929) were similar. However, the 2-breed crossbreds (−US\$0.20) had significantly lower replacement cost per day, because the replacement cost was distributed over more days in the herd compared with their HO herdmates (Table 9).

The 2-breed crossbreds (−13%) had significantly lower lifetime health treatment cost compared with their HO herdmates. Therefore, when lifetime cost was divided by days in the herd for each cow (Table 9), the 2-breed crossbreds averaged 29% lower daily health treatment cost compared with their HO herdmates.

Total daily expense was US\$11.84 for the 2-breed crossbreds and US\$12.19 for their HO herdmates (Table 9). The most important contributor to reduction of expenses for the 2-breed crossbreds was their lower replacement cost compared with their HO herdmates resulting from the longer days in the herd. The lower replacement cost was 57% of the total difference in expense for the combined 2-breed crossbreds compared with their HO herdmates.

The VR×MO/HO 3-breed crossbreds had 3% less daily income from production compared with their HO herdmates. However, the MO×VR/HO 3-breed crossbreds had 1% more daily income from production compared with their HO herdmates (Table 10). Daily calf value averaged US\$0.06 more for the combined 3-breed crossbreds compared with their HO herdmates. Lifetime cull value was higher for the VR×MO/HO 3-breed crossbreds (US\$944) and the MO×VR/HO 3-breed crossbreds (US\$953) compared with their HO herdmates (US\$814). However, the 3-breed crossbreds averaged 147 days longer in the herd compared with their HO herdmates. Therefore, the average cull value on a daily basis was US\$0.01 lower for the VR×MO/HO 3-breed crossbreds and +US\$0.03 higher for the MO×VR/HO 3-breed crossbreds and was not a major contributor to the difference in profit between the breed types. The combined 3-breed crossbreds had 1% lower daily income (US\$16.13) compared with their HO herdmates (US\$16.23). The 3-breed crossbreds had fewer lengthy lactations and calved back sooner each lactation compared with their HO herdmates. Therefore, the estimated feed intake for maintenance of the crossbred cows was lower because the crossbred cows had more days near peak production during their lifetimes compared with their HO herdmates.

Lifetime replacement expense was significantly lower for the VR×MO/HO 3-breed crossbreds (US\$1,887) and the MO×VR/HO 3-breed crossbreds (\$1,902) compared with their HO herdmates (US\$1,923) because of the younger age at first calving of the 3-breed crossbreds. Furthermore, the 3-breed crossbreds distributed costs over more days in the herd, and this resulted in a major reduction in daily replacement cost (−US\$0.21) compared with their HO herdmates.

Table 10. Daily income and expense that contributed to daily profit for VR×MO/HO and MO×VR/HO 3-breed crossbreds compared with their HO herdmates.

	Holstein	Difference from Holstein	
		VR×MO/HO	MO×VR/HO
Cows (number)	250	109	117
Income (US\$)			
Production	\$15.09	−\$.45**	+\$.10**
Calf value	\$.43	+\$.07**	+\$.05**
Cull value	\$.72	−\$.01**	+\$.03**
Total income (US\$)	\$16.23	−\$.38**	+\$.18**
Percentage difference from Holstein		−2%	+1%
Expense (US\$)			
Feed (during lactation)	\$5.39	−\$.16**	−\$.04**
Lactation overhead	\$4.20	−\$.04**	−\$.01
Replacement	\$1.60	−\$.26**	−\$.16**
Dry cow overhead (including feed)	\$.41	+\$.03**	+\$.01
Health treatment	\$.25	−\$.08**	−\$.09**
Breeding	\$.19	−\$.02**	−\$.02**
Total expense (US\$)	\$12.28	−\$.52**	−\$.33**
Percentage difference from Holstein		−4%	−3%

** P ≤ 0.01 significantly different from Holstein.

Lifetime health treatment cost was 26% less for the 3-breed crossbreds (US\$170) than for their HO herdmates (US\$229), and daily health treatment cost was US\$0.09 lower for the 3-breed crossbreds compared with their HO herdmates. Also, the lifetime breeding expense, which included semen, insemination fees, and supplies, was similar for the 3-breed crossbreds and their HO herdmates. However, average daily breeding expense for the 3-breed crossbreds (US\$0.17) was lower than for their HO herdmates (US\$0.19).

The 3-breed crossbreds (US\$11.84) had 4% lower daily expenses compared with their HO herdmates (US\$12.28). Difference for replacement cost was the most influential expense and was 48% of the total difference in expense between the combined 3-breed crossbreds compared with their HO herdmates.

All four of the crossbred breed types had significantly higher lifetime profit compared with their HO herdmates, and the difference ranged from +18% to +58% (Table 11). Both types of 2-breed crossbreds had significantly higher daily profit compared with their HO herdmates. The combined 2-breed crossbreds had 13% higher daily profit compared with their HO herdmates (Table 11). Likewise, the combined 3-breed crossbreds had 9% higher daily profit. This outcome may seem surprising because the 3-breed crossbreds had less fat + protein production compared with their HO herdmates. However, the lower expense of the 3-breed crossbreds compared with their HO herdmates resulted in an advantage for daily profit of the VR×MO/HO 3-breed crossbreds (+4%) and MO×VR/HO 3-breed crossbreds (+13%).

Table 11. Lifetime profit and daily profit for VR×HO and MO×HO 2-breed crossbreds and VR×MO/HO and MO×VR/HO 3-breed crossbreds compared with their HO herdmates.

	Holstein	Difference from Holstein	
		VR×HO	MO×HO
Cows (number)	640	376	358
Lifetime profit	\$2,842	+\$498†	+\$1,638**
Percentage difference from Holstein		+18%	+58%
Daily profit	\$3.74	+\$0.22**	+\$0.72**
Percentage difference from Holstein		+6%	+19%
	Holstein	Difference from Holstein	
		VR×MO/HO	MO×VR/HO
Cows (number)	250	109	117
Lifetime profit	\$2,823	+\$902*	+\$938*
Percentage difference from Holstein		+32%	+33%
Daily profit	\$3.95	+\$0.17**	+\$0.51**
Percentage difference from Holstein		+4%	+13%

† P ≤ 0.10, * P ≤ 0.05, ** P ≤ 0.01 significantly different from Holstein.

Sensitivity Analysis for Feed Intake

The actual feed intake of individual cows was not available for cows in this study because the herds did not have the ability to collect individual feed intake. Production and stage of lactation were the only factors used to estimate feed intake of individual cows for this study, and no potential differences in feed intake or feed efficiency of the breed types were taken into account. However, previous research compared ProCROSS® crossbreds to their HO herdmates for feed intake during the initial 150 days of lactation (Shonka-Martin et al., 2019). Daily feed intake was recorded, converted to dry matter intake, and analyzed. The ProCROSS® cows had 4.8% less dry matter intake in first lactation and 6.5% less dry matter intake in second and third lactation compared with their HO herdmates with absolutely no difference in fat + protein production (kg).

The breed type differences for feed intake from the previous study were applied to this study to estimate the feed intake of cows and then were converted to feed cost. The result was a much larger advantage in daily profit for both the 2-breed crossbreds (+US\$0.79 per day) and 3-breed crossbreds (+US\$0.66 per day) compared with their HO herdmates. The higher daily profit of the 2-breed crossbreds (+21%) and the 3-breed crossbreds (+17%) compared with their HO herdmates, including their potential advantages for feed efficiency, may better reflect the actual daily profit of the ProCROSS© crossbreds compared with their HO herdmates in this study.

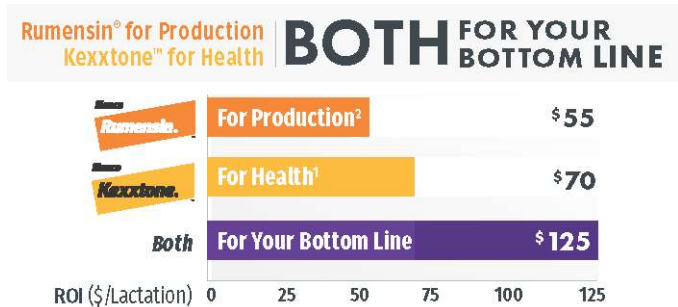
■ Breeds for Rotational Crossbreeding

ProCROSS© is a 3-breed rotational program, and three distinct breeds provides an average hybrid vigour of 86% across generations. Use of only two breeds for crossbreeding provides an average hybrid vigour of 67% across generations, and this is a 17% reduction of average hybrid vigour from a 3-breed to 2-breed rotation. Choosing three breeds to include for rotational crossbreeding should be based on breeds that 1) have highly-effective genetic improvement programs that emphasize important traits for improved profitability, 2) best complement each other for individual traits, and 3) provide a blended outcome across generations that is most appropriate for the environmental conditions of a herd.

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1. Bohary et al., 2016. Economic value of ionophores and propylene glycol to prevent disease and treat ketosis. (U) 57-733-740. Return based on a \$22 milk cost and 3:1 ROI. 2. Return based on \$0.21/kg of DM feed cost and a concentration of 16 ppm of Rumensin® in the diet. The label contains complete use information, including cautions and warnings. Always read, understand and follow the label and use directions. Rumensin, Kexxtone, and the diagonal bar logo are trademarks of Elanco or its affiliates. ©2021 Elanco. PM-CA-21-0111



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Tail Chalk Improves Estrous Detection when using Automated Activity Monitors

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A decrease in displaying estrous behavior has been noted and associated with high-producing animals. The aim of this study was to determine the association between physical activity, mounting behaviour and milk production in spontaneous estrus. A total of 1,127 estrus events from 376 Holstein cows were recorded. Activity was monitored continuously by an automated activity monitor (AAM) and mounting behaviour was monitored twice daily using tail chalk (TC) scores (1 to 3 scale, 3 was considered an alert). Ovarian ultrasonography was performed on the event day and 7d post-AI to determine true estrus alerts. Pregnancy was diagnosed at 30±3 d post artificial insemination (AI). Milk production was recorded and cows were classified as low (L) or high (H) using the median (39.5 kg/day). Activity was classified as High and Low by the median (230 heat indicator; AfiActII, Afimilk). The proportion of animals that showed mounting behaviour, increase in activity, or both were not different between H or L producers ($P = 0.27$) nor parity ($P = 0.75$). A higher proportion of cows were in true estrus when both tools alerted (AAM=65.3; TC=32.3; AAM+TC=88.6%; $P < 0.001$). Cows classified as High activity showed a greater proportion of TC alerts (1=30.2; 2=12.6; 3=57.2%; $P = 0.04$) than cows classified as Low activity (1=61.9; 2=15.0; 3=23.1%; $P = 0.04$). Cows with TC alerts tended to have a greater pregnancy/AI compared with cows that were only alerted on the AAM (AAM+TC=44.0±2.9; TC=41.0±7.3; AAM=37.0±2.9%; $P = 0.06$). In conclusion, estrous behaviour detected by TC or AAM was not impacted by milk production. The use of TC along with AAM increased true alerts and cows with TC alerts had higher pregnancy/AI than those that did not display standing to be mounting behaviour.

Take home messages: Tail chalk in companion with AAM can improve the efficiency of reproductive programs. Milk production did not affect estrous behaviour detected either by tail chalk or activity monitors.

Impact of Steam Pressure Processing Times on Nutrient Digestibility, True Protein Supply to the Small Intestine, and Predicted Milk Production of Faba Bean Seeds for Dairy Cattle

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Currently, the use of faba bean plant as alternative feed ingredient in cattle rations has gained more attention since high nutritional value varieties have been developed over the years. Thus, the purpose of this study was to provide supporting research on the effects of steam pressure processing (autoclaving) on nutrient digestibility, true protein supply to the small intestine, and predicted milk production of faba bean dairy diets. Seeds from CDC Snowbird variety were either kept raw or heated at 121 °C for 30, 60, 90, and 120 min. The experimental design was RCBD and the data were analyzed using the mixed model procedure with SAS software 9.4 (SAS Institute, Inc., Cary, NC, US) with significance declared at $P < 0.05$. The intestinal digestible crude protein increased sharply from 62 g/kg DM in raw seeds to 220 g/kg DM at 120 min of heating ($P < 0.01$). Whereas the total tract digestible starch gradually decreased from 322 g/kg DM in raw seeds to 182 g/kg DM at 120 min of heating ($P = 0.04$). The metabolizable protein based on NRC 2001 increased from 130 g/kg DM in raw seeds to 282 g/kg DM at 120 min of heating ($P < 0.01$). Lastly, the predicted milk production showed a gradual increase from 2.63 kg milk/kg DM feed in raw seeds to 5.73 kg milk/kg DM feed at 120 min of heating ($P < 0.01$). Different steam pressure times showed potential positive effects on the nutrient digestive behavior of faba bean seeds analyzed by prediction models. The present results will serve as the basis for complementary studies of alternative ingredients for dairy rations and for future application in the feed industry.

Giving Calves a Good Start: Lessons from the Maternity Pen

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■ Take Home Messages

- ▶ Understanding the natural behaviour of cows and calves around calving can provide insight into their care and management
- ▶ Dairy cows separate from the herd and find a secluded place to give birth when kept indoors or outdoors
- ▶ Allowing cows the ability to separate from herd mates may result in shorter labor
- ▶ Limited research has been done to understand natural calf behaviour in the first few hours and days after birth
- ▶ In nature, calves stay hidden with their dams for the first few days of life; they are later introduced to the herd within about a week of life
- ▶ We recommend future research to investigate the natural behaviour of calves in early life

■ Introduction

Research over the last ten years has investigated maternity pen design from the cows' perspective. This research has provided insight into preferred calving environments for dairy cows that allow for the performance of natural behaviours. This information can help inform dairy producers about practical ways to adapt their maternity areas to accommodate cow behaviour. The majority of maternity pen research has focused on the cows' experience in the few days before giving birth. By comparison, much less research has focused on the calf and the interaction between the cow-calf pair.

Dairy calves experience several challenges in the first few days and months of life, including separation from their dam, novel and sometimes isolated environments, novel feed and feeding methods and exposure to environmental stressors such as poor housing and ventilation. These factors likely contribute to a high risk of disease and death in pre-weaned calves. Traditionally, the dairy industry has focused on creating housing and management strategies for newborn calves that have been thought to limit pathogen exposure (e.g., separation from the dam and individual housing). However, new research is showing that more natural environments (e.g., prolonging contact with the dam and pair housing or small group housing with other calves) may not increase the risk of disease in calves (see reviews by Costa et al., 2016; Beaver et al., 2019; Meagher et al., 2019). Instead, providing calves a more natural social environment has benefits such as improved cognition and social skills. Very little research has investigated the calves' social environment in the first few hours and days of life.

For these proceedings, we will focus on reviewing research to date exploring the impact of the maternity area on the health and welfare of dairy cows and their calves. We will start with a review of what we currently know about the natural behaviour of cows and calves during the few days before and after birth, drawing from research using dairy cattle, beef cattle and other wild ungulates. We will then review the research on cow behaviour approaching calving, and the behaviour of the dam-calf pair in the first few days of life when kept in commercial settings. We will then discuss some promising research linking the design and management of the maternity area to cow and calf outcomes and will end with recommendations for future research.

■ The Behaviour of Cattle in Natural Environments

Cow Behaviour in Preparation of Birth

For the majority of mammals, the survival of young is dependent upon the care provided by mothers. Maternal behaviours include those expressed by mothers during late gestation in preparation for giving birth and directed towards offspring until young are weaned.

One of the first signs of maternal behaviour in wild ungulates and domesticated cattle is isolation from the herd to find a secluded birth site (Lidfors et al., 1994). Dairy cattle kept on a large rangeland isolated themselves from the herd as calving approached, but the degree of isolation was dependent on the resources available in the environment. Greater separation from the herd occurred when cows had to travel further distances to find a suitable calving site (e.g., dry, soft ground and overhead cover). Similarly, Flörcke and Grandin (2014) found that beef cattle managed on extensive rangeland were observed to separate 25 to 1,250 m from the herd to find a calving site, and a majority of animals moved more than 100 m away. The beef cows in this study sought hollows with sandy soil surrounded by small bushes and avoided open spaces to give birth.

Dairy cattle managed on pasture appear to have retained calving behaviours similar to those in extensively managed, natural environments. A recent study found that cows kept on pasture with access to natural cover (e.g., tall grasses and overhead trees), open pasture (no environmental cover), and a manmade barn (deep bedded with straw and overhead cover), calved either in the area with natural cover or the barn (Edwards et al., 2020). The choice of calving site was dependent on parity; heifers giving birth for the first time were more likely to calve in the pasture area with natural cover, whereas older cows were more likely to choose the barn for their calving site. It is unclear what drove the difference in calving location, but we speculate that previous experience and social status may have contributed to the animals' choice.

It is not clear why cattle and other ungulates choose to separate from the protection of their herd to find a secluded place to give birth. These behaviours are thought to be a strategy to improve offspring survival through reducing the likelihood of predation and disturbance from other cows. Other cows in the herd, particularly those preparing to give birth, may interfere with the development of the dam-calf bond. For example, Finger et al. (2014) found that beef cows preparing to give birth separated more from pregnant animals compared to non-pregnant animals before and after giving birth. For cattle that rear their young, such as wild ungulates and beef cattle, the formation of a bond with their calf occurs within hours of birth and is critical for calf survival.

Cow and Calf Behaviour After Birth

For ungulate species, there are two general styles of rearing described in the literature: 'hider' and 'follower' (Lent, 1974). Most species use a combination of these strategies to avoid predation of their newborn. For a 'hider', the offspring stays hidden or covered in secluded habitats for the first few days after birth while the mother grazes nearby. For a 'follower', the offspring stays close to the mother and is more active than a hider during the first few days after birth.

There is no research to our knowledge that has described the strategies used by domesticated dairy cows to rear their offspring in a natural setting. However, there has been some research with beef cows and feral populations of cattle that provide insight into post-calving behaviours in cattle. For example, Maremma cow-calf pairs (free-ranging cattle in Italy) perform both hiding and following behaviour during the first week after birth (Vitale et al., 1986). During the first three to four days of life, calves stayed hidden in bushes while their dams grazed nearby. Calves then followed their dams for the next few days, and finally rejoined the herd with the mother after about one week of life. Similarly, beef cattle kept on pasture reduce the amount of time spent with other animals on the day of calving, and gradually increase social contact with the herd over the span of one week after calving before fully rejoining the group with the calf (Swain et al., 2015).

■ The Maternity Pen

Designing the Maternity Area from the Cow's Perspective

The research described so far has focused on the behaviour of cattle and other ungulates as calving approaches in natural settings. It may not be practical to allow dairy cows to calve in large outdoor areas, such as large rangeland. However, we can use insights gained from studies in natural settings to design maternity areas that allow for expression of the cows' and calves' natural behaviours.

Indoor-housed dairy cattle have retained many of the maternal behaviours seen in cattle in outdoor settings, such as seeking shelter at calving and separating themselves from herdmates (Proudfoot et al., 2014a,b; Creutzinger et al., under review). For example, Proudfoot et al. (2014a) found that 79% of dairy cows in individual maternity pens calved behind a solid wall providing separation from a larger group pen compared to calving behind a gate where other cows were visible (Figure 1).



Figure 1. Options for creating an area of seclusion in individual maternity pens for dairy cows (the image on the left was from Proudfoot et al.(2014b) and the image on the right is courtesy of Vander Made Dairy, Ohio).

Creating opportunities for cows to seclude themselves at calving in group pens may be more challenging because of competition over resources. For example, Proudfoot et al. (2014b) found that cows were more likely to calve in a large shelter area of a maternity pen when they were kept individually (62% used the shelter to calve) compared with when they were kept with a partner (34% used the shelter to calve). Thus, when creating a space for cows to seclude themselves in a group maternity pen, it is important to make sure cows are able to access the provided resources for hiding.

Most of the recent research on creating hiding spaces for dairy cows at calving have used similar 3- or 4-sided shapes made of solid material. An alternative hide design was recently used in a study by Creutzinger et al. (2020); for this study we used a single-sided 'blind' in a group pen for six to ten animals (see Figure 2). This shape allowed more than one cow to use the hiding area at the same time but provided much less cover compared with other hiding spaces. We found that about 36% of cows chose to calve directly next to the blind, which was higher than the same area of an identical pen without a blind (14%). While not as many cows used this single-sided design as other designs, we only provided space for about two cows out of six to ten. Including more linear space in a single-sided blind may increase the number of animals that use the blind to give birth.



Figure 2. Options for creating a more secluded area for cows in group maternity pens. The photo on the left shows a single-sided ‘blind’ in a group maternity pen (from Creutzinger et al., 2020), and the photo on the right shows an ‘L’ shaped hide (from Zobel et al., 2020).

Indoor-housed dairy cows also seek separation from other cows as they approach calving. For example, Proudfoot et al. (2014b) found that, when pair-housed in a maternity area, cows separate from their pen-mates starting approximately 8 hours before calving. When cows were kept in groups of six to ten in sawdust-bedded packs with either low (~ 9.3 m²/cow) or high (~ 18.6 m²/cow) lying space, cows increased their distance from pen-mates as calving approached regardless of treatment (Creutzinger et al., under review). Cows given more space (~ 18.6 m²/cow) were farther away from other cows in the pen at the moment of calving compared with those given less space, suggesting that cows will use additional space to separate from other cows if provided.

Cow and Calf Behaviour in Maternity Areas

Cow and calf behaviour in the first few hours and days after calving are impacted by the animals' social environment in the maternity area. For example, cows kept in group maternity pens spent less time licking their calves during the 6 hours after calving compared with those kept in individual pens, likely because other cows in the group were also found to be licking their newborn (Edwards, 1983). In the same study, about 30% of calves born in group pens were nursed by a cow other than their mother. This behaviour, referred to as 'mis-mothering', can be reduced by providing the cow and her newborn a secluded space after calving (Jensen et al., 2019).

It is unclear how 'mis-mothering' behaviour impacts the cow and her calf, but it may be stressful for the new mother to have interference from other cows when she is trying to nurse and groom her newborn, and may also impact the colostrum quality of cows being nursed before giving birth. Thus, we recommend that cows either give birth in individual pens, or if cows calve in groups, the cow and her newborn should have the opportunity to seclude themselves. Alternatively, cow-calf pairs should be moved to a protected space shortly after birth to limit 'mis-mothering' behaviour.

There is also some evidence that newborn calves, similar to their dams, are attracted to more secluded areas in the first few hours after birth. Zobel et al. (2020) kept New Zealand dairy cows in outdoor paddocks with 'L' shaped hides (18:50 hides:cows; Figure 2) overnight during the few weeks before calving. Only about 20% of cows used the hides to give birth, but a majority of calves (73%) later moved themselves into the hides with their dams in the few hours after birth (~ 2.4 hours after birth).

The Impact of the Maternity Area on the Cow and Her Calf

The studies described above have focused on determining the preference and natural behaviours of cows and their calves around calving. Understanding animal preferences and behaviours can provide insight into what types of environments they like, and providing animals with choices is important for their welfare. We also acknowledge that other outcomes measurements, including health and affective states, may give us more information about how these environments affect the animals.

Our knowledge about the impacts of indoor environments that mimic more natural settings is limited; however, there has been some recent, promising work to show that maternity pens that allow greater space for separation may be beneficial for cows and their calves. For example, Creutzinger et al. (under review) found that cows provided more lying space (~18.6 m²/cow) and access to a single-sided 'blind' (Figure 2) before calving had the shortest duration of stage 2 labour compared with those kept with less space (~9.3 m²/cow) and no access to a blind. We speculate that stressors experienced by cows (e.g., an inability to isolate using environmental resources and distance to other animals) may prolong labour, as it would be beneficial for the cows to wait until they are in a more relaxed state before giving birth.

Another factor that may impact a cows' ability to progress through labour without disruption is human interaction. Cows are often moved from a group pen to an individual pen during and before labour, which may provide them with the seclusion they are looking for before giving birth. However, the interaction with farm staff may also be stressful to cows. Proudfoot et al. (2013) had farm staff move cows before labour, during early stage 1 labour (signs of suddenly tense and enlarged udder, raised tail or relaxed pelvic ligaments) and during late stage 1/early stage 2 labour (signs of viscous, bloody mucus or abdominal contractions). We found that cows moved during late stage 1/early stage 2 had longer labour (by approximately 30 minutes) and shorter lying time compared with those moved earlier. These results suggest that human interaction during labour and movement into a novel environment (the individual pen) may stall labour progress.

Another important outcome to measure in the maternity area for both the cow and calf is hygiene. Wet and dirty substrate in maternity areas potentially increases disease risk for cows and calves post-calving. For example, good hygiene in the calving area is considered critical for reducing the spread of Johne's disease between dams and calves for positive herds (Donat et al., 2016). Some research has also found associations between maternity area type (group or individual) and disease incidence in calves; however, these results may be confounded with pen hygiene (Svensson et al., 2003; Pithua et al., 2009). Calves born in individual maternity areas had lower risk of respiratory disease compared with calves born in group maternity areas (Svensson et al., 2003). In contrast, calves born in group maternity areas were not at greater risk of diarrhea, pneumonia, or morbidity compared with calves born in individual calving pens (Pithua et al., 2009). The link between maternity area type and calf disease is unclear but it may be related to frequency of pen cleaning and not maternity area type alone.

High stocking density in group maternity pens likely increases the risk of poor hygiene. Indeed, Creutzinger et al. (2020) found that cows provided less lying space (~9.3 m²/cow) had poorer hygiene compared with those provided more space (~18.6 m²/cow) when sawdust bedded packs were cleaned regularly (new sawdust added every other day and replaced every three weeks). We recommend that individual maternity pens are cleaned between calvings, and group bedded pack pens should have soiled material removed daily and fresh bedding added at least every other day to ensure calves are born into clean, comfortable areas. We also recommend that maternity pens do not double as hospital pens because this can also impact the transmission of pathogens to the calf.

■ Gaps in the Literature and Opportunities for Future Research

One of the knowledge gaps regarding maternity pen design is the calf perspective. A majority of the research in this review has focused on maternity pen design that provides cows the ability to express their normal behaviour before calving. We recommend researchers continue to explore natural behaviours in

calves during the first few days after calving. For example, following the work of Zobel et al. (2020), a future study could provide calves with their own ‘hiding space’ in maternity pens that they can use within the first few hours to days after birth. We do not encourage producers to socially isolate calves during this time because in nature the dam stays near the calf during this period. However, creating opportunities for calves to find seclusion in individual or group maternity pens may help them better cope with some of the challenges they face in the first few days of life.

A second area of research that we encourage is on alternative housing systems that allow for some cow-calf contact in early life (see Beaver et al., 2019; Meagher et al., 2019 for detailed reviews). For example, one of the most common patterns observed in wild ungulates and extensively managed beef cattle is the slow introduction of the calf and cow back in to the herd. Currently, cows are moved directly into fresh or high lactating pens, and calves moved into a nursery, sometimes within hours of birth. Research is encouraged to determine ways to mimic a more natural transition for both the cow and her calf into the herd by determining when the cow and calf would leave the maternity area on their own.

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Herd Health and Production Management Visits on Canadian Dairy Cattle Farms: Structure, Goals and Topics Discussed

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Regular veterinary visits to improve herd health and production management (HH&PM) provide opportunities for constructive veterinarian-farmer conversations and to direct management to proactively optimize animal health and welfare. However, little is known about the structure of HH&PM visits. In this study, our aims were to: describe HH&PM farm visit structure; determine discussed dairy-specific topics; and assess whether the focus of visits aligned with farmers' priorities. Audio-video recordings of 70 HH&PM farm visits by 14 Canadian veterinary practitioners were analyzed.

Consistent with farmers' priorities, the focus of visits was cow fertility; however, dairy-specific discussions were generally relatively infrequent, with only 17% of the HH&PM visit duration spent discussing dairy-specific topics, and short, lasting an average of 2 minutes. Veterinarians raised topics related to the whole herd more often than farmers. Most frequent topics included cow fertility, udder health, calf health/management, and transition diseases. However, answers to an open-ended question revealed that additional aims of many farmers were to receive information, have questions answered, and identify and discuss problems. A farmer's belief that HH&PM farm visits were 'absolutely' tailored toward their goals was positively associated with number of discussions per visit and their conviction that they 'always' voiced their wishes and needs.

In conclusion, opportunities to broaden the focus of HH&PM farm visits and improve veterinarian-farmer communication should be identified and veterinarians trained accordingly. As a result of specific training, veterinarians might become better equipped to add further value in HH&PM farm visits.

Effect of partial exchange of lactose with fat in calf milk replacer fed ad libitum on feed intake, feeding behaviour, and performance in dairy calves.

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Compared to whole milk, commercial calf milk replacers (MR) deliver relatively high levels of lactose and low levels of fat, resulting in lower energy density diets. This study evaluated the effects of partially exchanging lactose with fat in MR on feed intake, growth and feeding behaviour. Thirty-two male Holstein calves (2.1 ± 0.16 d of age, 46.4 ± 0.77 kg BW) were assigned to 16 blocks of 2 calves per block based on arrival date and serum IgG. Within each block, calves were randomly assigned to 2 treatments: a high lactose MR (HL, 17% fat; 44% lactose), or a high fat MR (HF, 23% fat; 37% lactose). Lactose was exchanged by fat on a weight-to-weight basis, resulting in a 6% difference in metabolizable energy (ME) density per kg of MR. The experiment was divided into 3 phases: P1 (0-35 d), P2 (36-56 d) and P3 (57-84 d). For the first 2 wk of P1, calves were in individual pens, fed their respective MR ad libitum through teat buckets, and provided access to water. At 14.2 ± 0.5 d of age, calves were group-housed (4 blocks/pen) for the rest of the study. In the group pens, calves were fed ad libitum MR, starter feed, chopped wheat straw, and water via automated feeders. During P2, calves were gradually weaned until complete milk withdrawal by 57 d and then monitored until 84 d (P3). Measurements included daily intakes and feeding behaviour (rewarded and unrewarded visits), weekly BW and body measurements, and biweekly blood samples. Increasing fat at the expense of lactose decreased MR intake during P1 by 15% ($P = 0.02$), whereas total starter intake was not affected. In P2, HL calves had 41% more unrewarded visits to the automatic milk feeder than HF calves. Despite dietary differences, ME intake remained comparable between treatments, while plasma cholesterol and non-esterified fatty acids (NEFA) levels were higher in HF calves because of the diet. Nevertheless, final BW (84 d) did not differ between treatments. Understanding the effect of energy source on energy metabolism may allow us to reach efficient growth performance, with the ultimate objective to support optimal lifetime performance.

Herd Health and Production Management Visits on Canadian Dairy Cattle Farms: Structure, Goals and Topics Discussed

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Impact of Concentrate Allowance and Cow Personality on the Behavior and Production of Dairy Cows Introduced to a Free-Traffic Automated Milking System

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Feed is typically used within an automated milking system (AMS) to motivate cows to voluntarily visit the milking unit. However, individual difference in personality traits between cows may also influence their willingness to milk voluntarily. The objective of this study was to determine the effects of concentrate allowance and the personality trait of boldness on the adaptability and production of dairy cows introduced to an AMS. Thirty-two Holstein cows (218±47 DIM; 1.6±0.8 lactations), with no previous exposure to an AMS, were designated as **Bold** or **Shy** based on previous observations of competitive behavior (displacements) at the feed bunk. Within personality trait category, cows were randomly assigned to an AMS concentrate allocation of either 6 (**High**) or 2 (**Low**) kg/d, on a dry-matter basis. Cows were trained to use the AMS over 72 h, being brought to the milking unit and encouraged to enter. After 72 h, cows were fetched to be milked when a minimum of 10 h had elapsed since the last milking. Data on milking activity and production were recorded for 9 wk following the initial training period. Compared to Low cows, High cows tended to have greater milking frequency (2.6 vs. 2.2 milkings/d; $P=0.1$) and had greater milk yield (35.0 vs. 29.9 kg/d, $P=0.01$). Compared to Shy cows, Bold cows tended to have their first voluntary milking visit earlier (5.4 vs. 7.3 d after introduction to AMS; $P=0.1$) and tended to have a shorter amount of time between their first voluntary AMS visit and their first 72 consecutive h with no fetches (15.5 vs. 33.0 d; $P=0.06$). Bold cows also tended to have greater milking frequency (2.6 vs. 2.2 visits/d; $P=0.1$) than Shy cows. Overall, these data indicate that allocating a greater amount of concentrate in the AMS, in a free-traffic setup, may promote adaption to voluntary milking. The results also suggest that Bold cows are more adaptable to an AMS than Shy cows.

Identifying the On-Farm Factors Associated with Elevated Free Fatty Acids in Bulk Tank Milk

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Elevated free fatty acids (FFA) in bulk tank milk are a recent concern in the dairy industry. High concentrations of FFA (> 1.2 mmol/100g of fat) are associated with undesirable milk characteristics, such as off-flavour, rancidity, reduced frothing ability, and inhibited milk fermentation and cheese coagulation. Previous research indicates that physical and chemical agitation cause the milk fat globule membrane to break and release triglycerides that dissociate into FFA. It is hypothesized that milking system type, pipeline diameter, and fat supplementation in the diet affect FFA. An observational case-control pilot study was conducted to identify on-farm factors associated with elevated FFA in bulk tank milk. 50 dairy farms in Ontario received a one-time visit to complete a survey, measure pipelines, and gather feeding information. Bulk tank FFA data were obtained from the Dairy Farmers of Ontario and used as the outcome variable for analysis. The mean bulk tank FFA level was 1.13 ± 0.3 mmol/100g of fat (range = 0.69 to 1.92 mmol/100g of fat). Univariable linear regressions indicated that smaller bulk tank capacity ($P=0.1$), decreased pipeline diameter ($P=0.09$), the absence of a plate cooler ($P=0.01$), and pasture access ($P=0.06$) were associated with FFA > 1.2 mmol/100 g milk fat. Multivariable analysis indicated that farms with a narrower pipeline diameter (< 2 inches) and farms that did not pre-cool milk before entry into the bulk tank were more likely to have elevated FFA; however, these variables only explained a small proportion of the variability in FFA (adjusted $R^2 = 22\%$). The results of this pilot study guide the development of a larger study that will include more farms and detailed measurements. Further, the initial results indicate that the risk of elevated FFA levels may be lessened with larger diameter pipelines and pre-cooling of milk.