

MANAGEMENT, SANITATION, AND ACCURACY OF AUTOMATED CALF FEEDERS

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## ABSTRACT

The objective was to summarize management practices, identify factors associated with bacterial counts, and describe the variation in total solids concentrations of milk or milk replacer in automated feeders. Six dairy calf operations in Virginia and 4 in Minnesota employing 1 to 2 sophisticated automated calf feeders were visited biweekly for 26 to 28 wk. An initial management survey was conducted for each farm. Observations on facilities, calf weights and heights, blood samples to estimate serum total proteins, treatment records, digital feeding behavior records, and milk or milk replacer (MMR) samples were collected at each visit. Additional milk replacer (MR) samples were collected for 4 wk pre- and post-circuit cleaning. Samples of MMR were plated on Aerobic Plate Count (APC) and Coliform Count (CCP) Petrifilms. Total solids concentration was estimated for MR samples by refractometry. Feeding plans varied widely between farms. Estimates of calf growth were near industry standards, but the proportion of calves receiving treatment was elevated. Least squares mean APC and CCP were 5.26 and 3.01  $\log_{10}$  cfu/ml for Virginia and 3.80 and 0.61  $\log_{10}$  cfu/ml for Minnesota. Circuit cleaning (CC) caused 13 and 16% log reduction in APC and CCP. However, more frequent CC/wk increased bacteria. Mixer/heat exchanger cleanings decreased bacteria during biweekly farm visits. Chlorine bleach reduced bacteria. Use of silicone feeder hoses increased bacteria. A quadratic effect of MMR liters delivered/d was observed; liters delivered > 147 L decreased APC. Automated feeders delivered 12.26% of MR samples > 2% over and 25.71% > 2% under target solids concentration.

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## LIST OF ABBREVIATIONS

<b>ADG</b>	Average daily gain
<b>ADHG</b>	Average daily growth in hip height
<b>APC</b>	Aerobic plate count
<b>BFV</b>	Biweekly farm visit sampling period (winter to fall 2014)
<b>BW</b>	Body weight
<b>CB</b>	Chlorine bleach
<b>CC</b>	Circuit cleaning
<b>CCP</b>	Coliform count
<b>CP</b>	Crude protein
<b>DET</b>	Detergent level
<b>DLCC</b>	Days since last circuit cleaning
<b>DM</b>	Dry matter
<b>HLMC</b>	Hours since last mixer/heat exchanger cleaning
<b>HTST</b>	High-temperature, short time
<b>IS</b>	Institute software
<b>KM</b>	Kalb Manager software
<b>LR</b>	Log reduction achieved by circuit cleaning
<b>LRAPC</b>	Log reduction of aerobic plate count
<b>LRCCP</b>	Log reduction of coliform count
<b>LSM</b>	Least squares mean
<b>MCHE</b>	Mixer/heat exchanger cleaning
<b>ME</b>	Metabolizable energy
<b>MMR</b>	Milk or milk replacer
<b>MMR L/d</b>	Daily liters of milk or milk replacer delivered by automated feeder
<b>MN</b>	Minnesota
<b>MR</b>	Milk replacer
<b>PBS</b>	Phosphate buffered saline
<b>PctLR</b>	Percent log reduction
<b>PLST</b>	Plastic hose
<b>PPCC</b>	Pre/post circuit cleaning sampling period (July to August 2014; VA farms only)
<b>pre-CC</b>	Collected immediately before circuit cleaning
<b>PWM</b>	Pasteurized waste milk
<b>RV</b>	Rewarded visits
<b>SIL</b>	Silicone hose
<b>SD</b>	Standard deviation
<b>SE</b>	Standard error
<b>URV</b>	Unrewarded visits
<b>VA</b>	Virginia
<b>VIN</b>	Vinyl hose

## **CHAPTER 1. MANAGEMENT OF AUTOMATED CALF FEEDER SYSTEMS**

### **ABSTRACT**

The objective was to summarize management practices regarding automated calf feeders on farms with systems currently in use. Six dairy calf operations in Virginia and 4 in Minnesota employing 1 to 2 sophisticated automated calf feeders were visited biweekly for 26 to 28 wk. An initial management survey was conducted for each farm. Observations on facilities, calf weights and heights, blood samples to estimate serum total proteins, treatment records, digital feeding behavior records, and milk or milk replacer (MMR) samples were collected at each visit. Feeding plans varied widely between farms, with peak daily allotments ranging from 7 to 16 L and feeding plan length ranging from 40 to 63 d. Mean number of calves per automated feeder was 25, which is less than half the maximum recommendation. On average, all farms provided more bedded space than the recommended 2.8 m<sup>2</sup>. Estimates of calf growth were near industry standards, with a mean average daily gain (ADG) across farms of 0.75 kg/d. Proportions of calves receiving treatment were elevated and variable between farms, ranging from 43.6 to 91.2%, with a mean of 61.7%. Data were compared from two software programs monitoring feeding behavior, showing inconsistencies between the two programs. Since few field studies on automated feeders have reported management practices and calf performance, producers with such systems can use this information to compare their calf programs to those evaluated in this study.

## **INTRODUCTION**

Automated feeders for preweaned dairy calves have become increasingly prevalent on dairy farms in the United States and Europe. Benefits associated with feeding, labor, and calf monitoring and wellbeing have contributed to the widespread adoption of these systems. The benefits, or conversely, the disadvantages of these feeding systems have not been researched.

As a relatively new form of technology in dairy calf rearing, management of automated feeders has not been thoroughly studied on commercial farms. As a result, recommendations regarding feeding plans, facility design, maintenance, and especially sanitation are not consistent between feeder suppliers. Producers with these systems commonly have limited resources to consult, often designing their own protocols with little guidance. This has led to much variation in management protocols between dairy calf programs.

The objective of this study was to describe management practices and calf performance observed on Virginia and Minnesota farms utilizing automated feeders.



## REVIEW OF LITERATURE

### *FUNCTIONALITY OF AUTOMATED FEEDERS*

Automated feeders function by recognizing individual calves that enter a feeding station, mixing the appropriate amount of liquid feed for that calf, and delivering it through a rubber teat. In the United States, there are two main types of automated feeders used on calf operations. The simpler of the two allows a producer to set maximum daily intakes for individual calves and provides a daily report for calf intakes. A simple feeder can handle up to 25 preweaned calves on the system at once, and costs less than \$3,500. The alternative type of automated feeder is commonly referred to as a sophisticated automated feeder and allows for more control over calf feeding plans, more detailed monitoring, and automated cleaning cycles. In addition to a handheld device used for controlling machine settings and monitoring individual calf feeding behavior, this system offers a software program that enables a producer to easily monitor individual calf progress throughout the preweaning period.

Custom feeding plans can be designed with a sophisticated automated feeder. Designated in feeding plans are maximum allotment of milk or milk replacer per day, minimum and maximum meal size, and solids concentrations. Daily feed allotment controlled by an automated feeder plan generally starts low, increases gradually each day, reaches a peak, and gradually decreases to encourage weaning. Because of the meal size settings, a calf can have a meal multiple times throughout the day. As an example, a calf may be allotted 8.0 L for the day with a minimum meal size of 2.0 L and a maximum meal size of 2.5 L. Since she is allowed to consume 8.0 L within 20 h (default time), she can earn meal credits at a rate of 0.4 L /h. Assuming she has no meal credit starting at 12:00 AM, she will have to wait until 5:00 AM to consume 2.0 L. If she does consume 2.0 L exactly at that time, she will have to wait until 10:00 AM to consume

her next meal. Following this pattern, she will consume 8.0 L total in 4 meals. Of course, there is much variation in feeding behavior between individual calves in terms of meal size and frequency throughout the day.

## ***FEEDING AND HOUSING***

### ***Frequency of Feeding***

In contrast to traditional calf rearing practices, automated feeders allow calves to consume more than two meals throughout the day, more closely reflecting the natural feeding patterns of calves. Hafez and Lineweaver (1968) found that calves performed 4 to 6 suckling bouts daily when left with their dam. Unless calves are near weaning, most feeding plans allow them to have at least this many meals throughout the day. It has been suggested that increasing meal frequency is beneficial to calf performance and health. Sockett et. al (2011) examined the effect of 3x/d feeding compared to 2x/d feeding of the same dry matter (DM) of milk replacer (MR) per day. At weaning, calves fed MR in three feedings were 1.70 cm taller, 4.70 kg heavier, and gained 0.09 kg more per kg of DM than calves fed twice daily ( $P < 0.01$ ). These calves also consumed 26% more calf starter prior to weaning ( $P = 0.0122$ ). Benefits of 3x/d feeding were also realized once these animals entered the lactation. Thirty-four of the 35 heifers fed 3x/d entered the milking herd, compared to only 28 of the 35 heifers fed 2x/d. Additionally, 3x/d calves produced an average of 515 kg more milk and freshened 16 d earlier than 2x/d calves (Sockett et al., 2011).

Ahmed et al. (2002) found an increase in abomasal luminal pH in calves fed 3, 4, or 8x/d compared to calves fed only 2x/d. It was concluded that the increased pH could contribute to lower incidence of abomasal ulcers, and thus explains one reason for improved performance in calves fed more than twice daily.

### ***Increased Plane of Nutrition***

It is not a requirement to feed large allotments of liquid feed to calves on automated feeder systems, however, these machines enable large volumes to be fed easily and with minimal additional labor. Traditional feeding practices in dairy calf feeding have been designed to restrict intake of liquid feed, to encourage early consumption of calf starter in order to stimulate rumen development (Davis and Drackley, 1998). Due to the traditional sizes of feeding equipment, calves fed via bottle 2x/d throughout the preweaning period are commonly limited to 3.8 to 7.6 L of liquid feed per day. A 45 kg calf consuming only milk or milk replacer requires 3.5 Mcal of ME for rate of gain of 600 g/d, equating to 5.6 L of 22:20 CP:Fat milk replacer per d (NRC, 2001).

Ad libitum access to milk results in higher intakes and gains of preweaned calves compared to conventional feeding rates of 8 to 10% BW as-fed. Jasper and Weary (2002) showed that calves on an ad libitum diet consumed an average of 8.8 kg milk/d, while calves fed at a rate of 10% BW per d consumed an average of 4.7 kg/d ( $P < 0.001$ ). Although ad libitum calves consumed 0.1 kg less solid feed per d during the preweaning period ( $P < 0.01$ ), their ADG was 0.3 kg greater during that period ( $P < 0.001$ ), and they were not different from conventionally fed calves in their incidence of illness over the entirety of the study or in their consumption of solid feed and ADG during and following weaning (Jasper and Weary, 2002). Thus, feeding high rates of milk supports increased weight gain, but is not detrimental to calf health or solid feed intake following weaning. Brown et al. (2005b) found that calves fed a 30:16 CP:Fat MR were 12.1 kg heavier and 2.4 cm taller at weaning, had 0.289 kg greater ADG, and gain:feed conversion 0.111 greater than calves fed a 21:21 MR at the same feeding rate ( $P < 0.01$ ). The same group of researchers found that this high protein MR diet also increased

mammary parenchyma development, suggesting implications for increased milk production later in life (Brown et al., 2005a).

Other researchers have investigated the long-term benefits of increased nutrient intake in preweaned calves. Raeth-Knight et al. (2009) showed that calves fed a 28:18 CP:Fat MR on an intensive feeding plan (0.68 kg of powder in 3.4 kg water during d 1 to 10, then 1.02 kg powder in 5.1 kg water during d 11 to 42) freshened 27.5 d earlier than calves fed conventional 20:20 MR (0.57 kg powder in 3.51 kg water during d 1 to 35) ( $P = 0.05$ ), suggesting that a higher plane of nutrition during the preweaning period may contribute to earlier onset of maturity. Soberon et al. (2012) conducted an observational study encompassing data from 1,868 total heifers. Increased preweaning ADG and weaning weight were associated with greater first-lactation yield ( $P < 0.03$ ) (Soberon et al., 2012). Nutrient intake was also correlated with lactation performance; for each additional Mcal of milk replacer consumed above maintenance requirements, milk yield increased by 235 kg in the first lactation ( $P < 0.01$ ) (Soberon et al., 2012).

### ***Group Housing***

In order to economically manage an automated feeder system, calves are usually housed in groups, rather than individually. In the last several decades, group-housing systems have been explored by US dairy producers (LeBlanc et al., 2006). While group housing systems have traditionally been avoided by dairy producers as an attempt to mitigate spread of disease between calves, there are benefits to these systems, especially in regard to calf behavior. Calves housed singly spent less time playing than calves housed in pairs or with their dam ( $P = 0.04$ ) (Duve et al., 2012). While play behavior has not yet been shown to be directly related to calf health, it may improve animal wellbeing, especially since calves have been observed to perform this behavior in semi-wild environments (Reinhardt and Reinhardt, 1982; Vitale et al., 1986).

Housing calves with companions also may reduce levels of stress during novel events or entrance into novel locations. Warnick et al. (1977) demonstrated that calves housed in groups of 6 exhibited less anxiety to an open field test, measured by the number of squares calves entered in a circular area, compared to calves housed individually ( $P < 0.05$ ). During a social novelty test conducted by De Paula Vieira et al. (2012), individually-housed calves defecated more and interacted less with unfamiliar calves than those calves that were previously housed in pairs ( $P \leq 0.05$ ). Duve et al. (2012) showed that calves housed individually struggled more during blood collection than calves housed in pairs or with their dam ( $P = 0.01$ ).

Weaning is a particularly stressful event for all dairy calves. Reduced stress in response to novel environments or pen-mates exhibited by group-housed calves may allow them to cope with weaning better than individually-housed calves. De Paula Vieira et al. (2010) compared responses of pair-housed to individually-housed calves around weaning, which involved calves being relocated to a pen with unfamiliar calves and feeding systems. Pair-housed calves spent less time vocalizing and consumed more grain postweaning ( $P < 0.01$ ), and began consuming starter immediately, 2 d before individually-housed calves (De Paula Vieira et al., 2010). These results imply that calves face less of an intake setback immediately postweaning if previously housed with a companion.

Feeding behavior is also affected by the presence of pen-mates, with the element of competition likely being involved. Babu et al. (2004) observed that solid feed intake was higher in calves housed in groups of 6 than in individually-housed calves at 2, 4, and 8 wk of age ( $P < 0.05$ ). Additionally, group-housed calves consumed their bottle-fed milk more quickly than individually-housed calves, with significant differences observed at 3 of the 8 feedings recorded and numerical differences at all feedings recorded during the preweaning period (Babu et al.,

2004). From this, it can be concluded that group-housed calves are more aggressive eaters than individually-housed calves.

### ***CALF MANAGEMENT IN AUTOMATED FEEDER SYSTEMS***

Disease detection is a challenge in any calf feeding system, with 58 and 56% sensitivity rates for enteritis and pneumonia (McGuirk, 2008). Because calves are group-housed in automated feeder systems, they may receive less attention needed to quickly identify illness compared to calves that are individually-housed. However, automated feeders monitor calf feeding behavior to inform producers of individual intakes, drinking speeds, and visits to the feeder. This information can be used to supplement visual monitoring of calves. In an experiment on 2 farms utilizing automated feeders to feed 5.6 to 8.1 L of MR per day, Svensson and Jensen (2007) showed that healthy calves performed on average 20 unrewarded visits (URV) per day, while sick calves performed 16 ( $P < 0.01$ ).

Borderas et al. (2009) also identified feeding behavior measurements associated with illness in calves in a study comparing calves on low or high milk or milk replacer (MMR) allotments. Sick calves allowed  $\geq 12$  L/d reduced their visits to the feeder up to 2 d prior to illness detection and reduced MMR intake, visits to the feeder, and visit duration following illness detection ( $P < 0.05$ ). In contrast, calves allowed 4 L/d only changed their behavior by reducing visit duration on the day illness was detected and the following 3 days ( $P < 0.001$ ) (Borderas et al., 2009). The calves on the low allotment plan did not change their intake levels even after becoming ill, indicating that they remained hungry despite their illness. To support this idea, calves in the high allotment group continued to consume approximately 8 L/d during illness (Borderas et al., 2009). These results imply that it is easier to recognize sick calves with

automated feeding behavior measurements if calves are fed a high compared to a low MMR allotment.

In healthy preweaned calves, MMR allotment has been shown to affect feeding behavior as well. Jensen (2006) found that calves fed a high allotment of 6.4 L/d and 8.0 L/d for Jersey and large breed calves, respectively, made more rewarded visits (RV) and fewer unrewarded visits (URV) than calves fed a low allotment of 4.8 L/d and 3.8 L/d ( $P < 0.001$ ). Low allotment calves spent more time in the feeding stall after their allotment was reached and more than twice the amount of time on each URV ( $P < 0.001$ ) (Jensen, 2006). Limit-fed calves on automated feeder systems are more likely to spend their time occupying the feeder while not ingesting MMR compared to calves fed high allotments. This is an inefficient use of time for the calf and the feeding system; the calf could otherwise be resting or consuming dry feed, and the feeder could be otherwise feeding calves that are entitled to a meal.

Group size also has an effect on calf feeding behavior. In an automated feeder study comparing groups of 12 or 24 calves, Jensen (2004) showed that calves in the large group consumed MR faster and in fewer visits than calves in the small group ( $P < 0.01$ ). It is common for farms employing automated feeders to enroll more than 24 calves on a feeder. To reduce inefficient feeder occupancy time in larger groups, calves should be allowed a sufficient maximum meal size. Jensen (2004) showed that calves allowed to consume 25% of their daily allotment in a single portion spent less time in the feeder and left the feeder sooner after they completed their meal compared to calves that could only consume up to 12.5% in one portion ( $P < 0.001$ ). Increasing maximum meal size is an especially important management practice for older calves, since calves naturally consume their feed in fewer meals as they age (Jensen, 2009).

Drinking speed is monitored by automated feeders for individual calves, and may be used by producers to identify calves that are drinking more slowly than their baseline speed. However, limited research has been conducted to relate this measurement to calf health. Babu et al. (2004) found that with age, drinking speed increased as a result of calves consuming larger volumes with each mouth movement. Drinking speeds may differ between calves and depend on the diameters of feeder hoses and nipple openings. Jensen (2004) reported mean drinking speeds of 0.301 to 0.397 L/min on automated feeders.

### ***CHALLENGES WITH AUTOMATED FEEDERS***

Many challenges associated with automated calf feeders are related to group housing. Perhaps the most obvious is the risk of disease. In a study examining calf mortality rates on Norwegian dairies, Gulliksen et al. (2009) showed that group-housed calves (not necessarily fed by automated feeders) had a higher risk of mortality within the first month of life compared to individually-housed calves (hazard rate = 1.5). Svensson et al. (2003) found that calves fed by automated feeder and housed in groups of 6 to 30 had more severe cases of diarrhea and were more likely to develop respiratory disease than individually-housed calves ( $P < 0.001$ ;  $P < 0.05$ ). Increased group size also causes greater risk for disease, demonstrated in a study by Svensson and Liberg (2006) that showed calves on automated feeders in pens of 6 to 9 had a lower risk of respiratory disease than calves in groups of 12 to 18 ( $P < 0.0001$ ). Brscic et al. (2012) also found that veal calves housed in pens of  $> 15$  had more risk of respiratory disease than calves in pens of  $\leq 6$  ( $P < 0.05$ ).

Nose-to-nose contact and shared feeding equipment increase the chance of disease transmission between calves in group housing compared to calves in traditional housing systems. *Salmonella* spp. and other enteric pathogens may be transmitted via salivary secretions, so



sharing nipples, waterers, and troughs increases the risk of disease transmission (McGuirk, 2008). Enteric pathogens can be transmitted via fecal-oral route as a result of commingling animals, contaminated bedding, and self-grooming (McGuirk, 2008). Since the incubation period for most enteric pathogens is 12 to 120 h, diarrhea that occurs within 5 d of life is usually caused by pathogens originating from the maternity pen (McGuirk, 2008). Infections that occur after 5 d of entering the group pen can thus be attributed to a source within the calf pen.

Respiratory disease is usually caused by viral infections originating from older calves, transmitted either from contact or aerosol (Svensson and Liberg, 2006). Exposure to older pen-mates and poor ventilation therefore increase risk of respiratory disease in group-housed calves. Properly designed ventilation in calf barns is critical for respiratory health. Ventilation systems should have air exchange rates of 0.57, 1.70, and 3.68 m<sup>3</sup>/min during cold, mild, and hot weather, respectively (Hohmann, 2008). Bacterial concentrations in the air of calf barns can be measured to assess air quality and risk of respiratory disease. Bacterial counts of < 30,000 cfu/m<sup>3</sup> are indicative of proper ventilation, while counts of > 100,000 cfu/m<sup>3</sup> are associated with enzootic calf pneumonia (Nordlund, 2008).

Due to its effects on calf behavior, feeding, and disease transmission, group size is a management factor that requires attention in automated feeder systems. According to Hohmann (2008), a Förster Technik feeder with 2 feeding stations can feed up to 40 to 60 calves. On systems with 4 stations and feeding pumps, up to 100 may be fed with 1 feeder. In order to provide enough space for walking and resting, and to reduce disturbances from other calves, each calf should have access to at least 2.8 m<sup>2</sup> of bedded space (Hohmann, 2008). With these requirements, space is often more limiting than the feeding capacity of automated feeders. Producers should be aware of the feeding competition and increased risk of disease associated

with large groups of calves, and should design portion size, bedding, cleaning, and ventilation practices accordingly.

Automated calf feeders require routine maintenance for success of the calf program. Unfortunately, feeder management protocols are often inconsistent between dealerships of similar machines, and producers often have limited resources to consult.

Certain maintenance and cleaning practices require automated feeders to be inactive. Such practices are ideally performed during times that calf feeding activity is low. Jensen (2004) identified 06:00-08:00 and 18:00-20:00 as times during which the highest proportion of visits to an automated feeder were made. Conversely, the least activity occurred 22:00-03:00, with another lull in activity occurring mid-morning. It is unclear whether this feeding activity was stimulated by the presence of research staff or natural diurnal feeding activity. Vitale et al. (1986) observed the highest number of suckling bouts in semi-wild calves at 09:30 and 15:30. The difference between results presented by Jensen (2004) and Vitale et al. (1986) may be due to differences in day length, but also likely reflect calves' response to human presence in the automated feeder study.

### ***ECONOMIC FEASIBILITY***

Heifer rearing is a large investment, accounting for 20% of total dairy operation expenses (USDA, 2007). Expenses associated with raising calves have more than doubled per animal from 1998 to 2008, with much of the increase attributed to labor costs (Hohmann, 2008), which make up approximately 26% of the cost of raising calves to weaning (Karszes, 2013). Manual labor needed for traditional calf rearing, such as hand-mixing MR, feeding individual calves, and washing feeding equipment, is performed mechanically with automated feeders.

In a study comparing automated feeder systems to traditional individual calf feeding, Kung et al. (1997) examined labor requirements in both systems. Calves fed individually required 10 min of labor per d, while calves fed by automated feeder ( $\leq 15$  calves per feeder) required  $< 1$  min per day. Using this finding, a 7-wk feeding plan for an automated feeder would require approximately 49 min/calf, while 8.2 h/calf would be required in a traditional feeding system. At a labor cost of \$10/h, a facility feeding 100 preweaned calves/yr would incur a labor cost of \$820/yr with an automated feeder (feeding ~14 calves on 1 feeder) \$8,200/yr with traditional feeding. At a cost of approximately \$22,000 (Bentley and Paulson, 2012), an automated feeder would pay for itself after 3 years of use [ $\$22,000/(\$8,200-\$820)$ ]. This of course, does not consider installation or maintenance costs. Annual ownership cost on feeder purchased for \$22,000 with cash equates to \$3,614.29/yr (useful life = 7 yr; salvage value = 15% purchase cost; straight line depreciation).

## **MATERIALS AND METHODS**

This study was approved by the Virginia Tech Institutional Animal Care and Use Committee (#13-180).

### ***FARM SELECTION***

Six Virginia and 4 Minnesota dairy farms were enrolled in the study. Farms were eligible if they used at least 1 sophisticated automated feeder (Förster Technik, Engen, Germany) and fed a high plane of nutrition (peak daily allotment of  $\geq 7.0$  L milk or milk replacer at a concentration of  $\geq 12\%$  solids) in their preweaning programs. Automated feeders on all farms were equipped with at least one of two software programs. Kalb Manager (KM) is a program designed by Förster Technik that summarizes daily calf feeding behavior data. Farms with KM had installed the program prior to the study for their own use. Institute software (IS) supports more extensive data collection on feeding behavior and machine activity at specific time points, outputting 3 reports labeled “feeder,” “portion,” and “service.” Institute software was installed on farms by research personnel.

### ***DATA COLLECTION***

An initial survey of management practices regarding calf health, calf feeding, and automated feeder protocols was conducted by research personnel. MN farms were visited by the same University of Minnesota researcher weekly for approximately 26 wk. VA farms were visited by the same Virginia Tech researcher biweekly for approximately 28 wk.

A routine survey on facility conditions was completed at each visit. Cleanliness of feeders and quality of bedding, calf starter, and drinking water were scored on a subjective scale of 0 to 3, with 3 being the highest quality, and observations on ventilation and temperature were

recorded. Calf treatment and mortality records, maintained by farm staff, were collected at each visit. Calf feeding behavior records from KM and IS output were also collected.

During VA farms visits, weight was estimated by heart girth measurement and hip height was measured on calves within approximately 5 d of entering the feeder system or 5 d of being weaned. Weight was estimated for MN calves using the same criteria. One exception to this protocol existed; at the Virginia Tech Dairy Center (Farm VA-6) calf weights were measured with a digital scale and hip height was measured at birth and monthly as a routine practice of that farm. VA research personnel were present for the majority of these monthly weighings.

Approximately 5 ml of blood was collected from calves 1 to 7 d of age via jugular venipuncture, transported on ice, and refrigerated for a maximum of 3 d. In VA, samples were centrifuged at 2000 rpm for 30 min at 15 °C to separate serum. An optical serum refractometer (VEE GEE Scientific, Inc., Kirkland, WA), calibrated with distilled water prior to each session was used to estimate serum total proteins. In MN, samples were centrifuged at 2000 to 3000 rpm for 10 min at 15 °C. A digital serum refractometer (MISCO Refractometer, Solon, OH), calibrated with distilled water prior to each session was used to estimate serum total proteins.

Each farm had at least one form of feeder software, i.e. Kalb Manager (KM) or Institute (IS), and 7 farms had both software programs. Daily IS data were consolidated by farm by record type (i.e. feeder, portion, or service report) in Microsoft Excel. Daily milk or milk replacer (MMR) consumption, number of calves enrolled, rewarded visits (RV), and unrewarded visits (URV) were summarized for later use.

## RESULTS AND DISCUSSION

### *FARM MANAGEMENT*

Table 1 summarizes management practices for each farm that participated in the study. The lactating herd size of study farms ranged from 110 to 850 cows, with an average of approximately 370. Most herds were larger than the average of 148 and 128 cows for VA and MN dairy farms (Progressive Dairyman, 2014). One farm (MN-2) was a heifer growing facility receiving calves from 4 different herds. Regarding the calf programs, each farm housed calves in group pens that allowed access to one of two feeding stations per automated feeder. Seven farms managed pens with dynamic groups, meaning calves first entered a pen designated for young calves, then moved to a pen for older calves partway through the preweaning period. The remaining farms utilized an all-in/all-out method in which new calves entered a pen until it became full, at which point a different pen was filled. Calves were intended to remain in the same pen throughout the preweaning period in these systems. The all-in/all-out method is advantageous to calf performance, as it allows for pens to be completely sanitized between groups and may reduce disease transmission from older to younger calves. Pedersen et al. (2009) found that calves housed in stable groups of 6 gained 0.6 kg more per d ( $P = 0.0249$ ) and had fewer than half the scours and respiratory disease events ( $P < 0.01$ ) compared to calves in dynamic groups of 6.

Calves were backgrounded prior to entering the automated feeder system, during which they received colostrum and milk or milk replacer (MMR) individually. The age at which calves were enrolled on the feeder system and entered group pens varied between farms. Initial survey responses showed that age of enrollment ranged from 1 to 14 d, with an average of 6 d. Research on age of enrollment to automated feeders has not been published, but industry recommendations

exist. Hohmann (2008) recommended doing this at 5 to 7 d of age, whereas Earleywine (2010) recommended 6 to 12 d, while considering a calf's health and ability to drink vigorously.

The mean number of calves per automated feeder during farm visits ranged from 11 to 36, with an overall mean of 25 (12 to 13 per pen/feeding station) across farms. The number of calves per feeder was lower than the maximum recommendation of 60. However, most research on automated feeders has focused on smaller groups of calves, typically fewer than 12 calves per feeder. One study by Jensen (2004) compared behavior of calves housed in groups of 12 or 24, with 1 feeder in each group. Calves in the large groups appeared more competitive. Rate of milk ingestion was 0.397 and 0.301 L/min for calves in large groups and small groups, respectively ( $P = 0.04$ ). As a result, calves in the large group spent 4.33 fewer min/d on rewarded visits ( $P < 0.001$ ), improving the efficiency of the feeder and potentially allowing each calf more resting time.

The estimated average area per calf per feeder ranged from 3.1 to 15.2 m<sup>2</sup>, with a mean across farms of 7.4 m<sup>2</sup>. Space allotment was adequate, as the recommendation of 2.8 m<sup>2</sup> was typically met on all farms. Færevik et al. (2008) showed that group-housed 100 kg and 150 kg calves spent less time lying simultaneously and lying recumbently when allowed 0.75 and 1.00 m<sup>2</sup> compared with > 1.25 and > 1.50 m<sup>2</sup> ( $P < 0.05$ ). With space allotments several times higher than those tested by Færevik et al., it can be concluded that most calves in the current study were provided with sufficient space to encourage rest.

### ***FACILITY CONDITIONS***

Farms were visited from February to November 2014. At each farm visit, automated feeders and surrounding areas were scored on cleanliness on a scale of 0 to 3, with 3 being the cleanest score. Feeder mixers, hoppers, feeder hoses, outside and surrounding areas of feeders,

feeding stations, and milk replacer handling scored 2 and 3 the majority of the time. The largest proportion of mixer hose scores were 1. This silicone hose was almost never replaced or cleaned by hand on any study farm. Bedding, calf starter, and drinking water were also scored on a similar scale. The majority of calf starter scores were 3 and bedding and water scores were 2 (Table 2).

Temperature was recorded in calf barns and automated feeder rooms at visits. Barn temperature ranged from -26.1 to 30.0 °C, with a mean of 17.2 °C. Room temperatures ranged from 7.0 to 32.4 °C, with an overall mean of 20.8 °C.

### ***MILK AND MILK REPLACER FEEDING***

Researchers did not alter feeding plans on study farms, and a wide variety of programs were implemented. Feeding plan length (spanning day of automated feeder enrollment to weaning) ranged from 40 to 63 d, with an average of 51 d. Peak daily allotment ranged from 7 to 16 L, with a mean of 8.9 L. Two farms (MN-3 and MN-4) fed pasteurized waste milk (PWM) or a combination of milk replacer (MR) and PWM (Table 3). The other farms fed only MR, with some farms changing milk replacers several times throughout the study. Crude protein content of MR ranged from 22 to 27% DM, and fat ranged from 10 to 20% DM. The amount of MR powder added to 1 L water ranged from 143 to 165 g, with a mean of 153 g. Except for VA-1, all farm feeding plans maintained a constant concentration throughout the preweaning period.

A misconception of sophisticated automated feeders is that they deliver 500 ml portions of reconstituted MR. On the contrary, a feeding plan set to deliver 150 g powder to 1 L water will actually deliver a 575 ml portion, consisting of 75 g powder and 500 ml water. This is widely misunderstood by producers and consultants that primarily monitor intake by volume. Rather, MR powder intake should be used to monitor intake.



## ***CALF PERFORMANCE***

Calf growth estimates by farm are listed in Table 4. Mean estimated ADG ranged from 0.57 to 0.90 kg between farms, with a mean of 0.75 kg across farms. Jasper and Weary (2002) found that calves fed ad libitum milk (mean intake of 8.8 kg/d) had preweaning ADG of 0.78 kg, while conventionally fed calves (mean intake of 4.7 kg/d) had ADG of 0.48 kg ( $P < 0.001$ ). In comparing the effect of feeding ad libitum whole milk to ad libitum 24:13 CP:Fat MR, Moallem et al. (2010) found preweaning ADG of 0.81 and 0.73 kg for whole milk and MR, respectively ( $P < 0.01$ ). Therefore calves on the study farms performed nearly at the level of calves receiving ad libitum milk or milk replacer. At birth weights of approximately 42 kg, calves gaining 0.75 kg/d would double their body weight within an 8 wk period, a common goal in liquid feeding programs (Perdomo and Santos, 2011).

Mean gain in hip height was 0.18 cm/d, which was slightly lower than the 0.20 and 0.25 cm/d observed by Sockett et al. (2011) for 2x/d and 3x/d feeding. In a study comparing 2 different weaning methods, Khan et al. (2007) found a large difference in rate of hip height growth between treatments: 0.15 and 0.30 cm/d on milk feeding rates of 10% BW plus conventional weaning and 20% BW plus step-down method of weaning, respectively. Mean hip height for calves on VA farms (excluding VA-6, since those calves were measured under different age criteria) within 5 d of weaning was 90.9 cm, comparable to mean hip heights for weaned calves 49 d of age which were 83.4 and 94.5 cm for conventional and step-down weaning reported by Khan et al. (2007).

Table 5 shows a summary of estimated serum total proteins. Mean estimated serum total proteins ranged from 5.28 to 6.72 g/dL, with a mean of 5.84 g/dL across farms. Failure of passive transfer is defined as serum IgG levels of  $< 10$  g/L, estimated at serum protein levels of  $<$

5.2 to 5.5 g/dL (Deelen et al., 2014). In a study examining 400 serum samples from calves on commercial dairy farms, Deelen et al. (2014) found a mean serum total protein concentration of 6.0 (SD  $\pm$  0.8) g/dL, comparable to the results of the current study. In the initial survey, VA-1 and MN-2 reported regular monitoring of serum total proteins, and VA-6 developed this protocol after the start of the study. Although an inexpensive management practice, only 2.1% of US dairy operations routinely monitor serum total proteins in calves (USDA, 2007).

Table 6 summarizes treatment and mortality rates. According to treatment records maintained by farm staff, the proportion of calves receiving treatment ranged from 43.6 to 91.2%, with an average of 61.7% across farms. These rates are high compared to other reports on morbidity, likely due to disease risk associated with group housing. The USDA (1994) reported that by the fifth week of life, 37.9% preweaned heifers received treatment at least once, and by 8 weeks of age 27.2 and 8.9% of calves had been diagnosed with scours and respiratory disease, respectively. Mortality ranged from 0.0 to 6.4%, with an average of 2.0% across farms. This is an improvement in comparison to the national mortality rate of 7.8% for preweaned heifers (USDA, 2007). The proportions of calves receiving treatment were elevated on study farms, but mortality rates were surprisingly low, perhaps due to adequate nutrition aiding in recovery or improved calf monitoring from feeding behavior data reported by automated feeders.

Researchers did not have control over mortality records in the current study, therefore it is also possible that low mortality rates reflect an inability of some farms to accurately record all calf deaths.

### ***SANITATION MANAGEMENT***

Sanitation management of automated feeders is summarized in Tables 7-8. Researchers did not interfere with established cleaning practices. Farms varied from 1 to 7 circuit cleanings

per wk and 2 to 4 mixer/heat exchanger cleanings per d. Cleaning agents used in circuit cleaning included chlorinated alkaline detergent (sodium hypochlorite and sodium hydroxide), alkaline detergent (sodium hydroxide), peroxide/acid mix, and acid detergent. Levels of detergent used in circuit cleaning ranged from 5 to 25 ml per L water, with a mean of 13 ml. Four farms used additional chlorine bleach during circuit cleaning. Three farms used silicone, 3 used plastic, and 4 used vinyl feeder hoses.

### ***SOFTWARE DISCREPANCIES***

Feeding behavior data collected automatically from IS and KM during the entirety of the study, including days when MMR samples were not collected are summarized in Table 9.

Within-machine correlations between KM and IS records of daily MMR L delivered (MMR L/d), calves enrolled, rewarded visits (RV), and unrewarded visits (URV) for biweekly farm visit (BFV) data are shown in Table 10. The number of calves enrolled was least correlated between the two software programs. This may be explained by differing methods in how each program counts a calf. For IS, calf numbers were calculated daily by summing the number of individuals who were recorded performing an activity. This could include calves that are weaned, and it would exclude calves that do not enter the feeder that day. On the other hand, KM, keeps a log of the calves currently enrolled, and will count calves that do not enter the feeder on a given day. Although the software programs differed in this measurement, this is a physically countable number that a producer should be able to easily obtain without consulting software.

The daily MMR L delivered had the second highest correlation between IS and KM. While IS and KM are independent software programs, they obtain their information from the same machines, and both measure milk consumption in the same way. Therefore, the differences in MMR L/d records are largely due to disconnections between the machine and the software.

This is much more likely for KM than IS, since KM operates on a different device, typically a separate computer. On multiple farm visits, observations were made of failures of machine data to refresh KM reports or of malfunctions with laptops. Figure 1 shows a high incidence of negative values when IS is subtracted from KM to identify differences in records, suggesting that KM was typically the failing software program, especially for RV and URV. However, IS appears to have failed on multiple occasions as well.

Kalb Manager software is used by producers to view daily feeding behavior data for individual calves, whereas Institute software reports all individual events without summarizing, making it less practical for management use. The inconsistencies between software records in the automated feeders on the study emphasize the need for visual monitoring of calves. Producers should use digital and visual monitoring in combination to identify calves in need of treatment.

### ***CONCLUSION ON AUTOMATED FEEDER MANAGEMENT***

While all study farms employed similar automated feeders in preweaning calf rearing, a variety of management practices in facility management, feeding, and sanitation were observed. Although differing between farms, calf ADG and weaning hip height were near those reported by field studies for calves fed on a high plane of nutrition. However, treatment rates were high, likely related to elevated disease risk in group housing. Digital monitoring of calves was not completely reliable, and should be used in combination with visual monitoring to assess calf health.

The observations reported here should be considered by dairy calf producers with automated feeder systems. While management factors presented in this chapter were not compared to calf performance, producers may benefit from learning of practices used on other

farms, such as pen management and feeding plans. Additionally, producers using automated feeders can compare their calf weight, height, and health measurements to those presented here.

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Table 1. Farm management summary

Farm	Milking herd (N)	Mean number of calves per feeder <sup>1</sup> (machine 1, machine 2)	Mean resting area per calf (m <sup>2</sup> ) <sup>2</sup>	Pen management	Bedding	Age of enrollment (d) <sup>3</sup>
VA-1	310	22	11.4	Dynamic	Sawdust/straw	1-3
VA-2	160	11	15.2	Dynamic	Sawdust/straw	3
VA-3	110	18	7.5	Dynamic	Wood chips	5
VA-4	310	26	6.2	Dynamic	Stalks/sand	4-5
VA-5	850	28, 24	6.7, 5.6	Dynamic	Sawdust/straw	5
VA-6	210	18	10.0	Dynamic	Sawdust/straw	3-7
MN-1	186	31	5.4	Dynamic	Sawdust/straw	3-5
MN-2	N/A <sup>4</sup>	31, 32	7.7, 7.4	All-in/all-out	Straw	10-12
MN-3	780	36, 32	4.3, 4.8	All-in/all-out	Sawdust/straw	5-10
MN-4	400	36, 35	3.1, 3.2	All-in/all-out	Straw	10-14

<sup>1</sup>Recorded by researcher during farm visit; may include weaned calves

<sup>2</sup>Calculated by totaling area in pens for each machine and dividing by mean calves on machine

<sup>3</sup>Calf age at entrance to automated feeder system; reported in initial surveys

<sup>4</sup>Heifer grower receiving calves from 4 herds

Feeder item	Score			
	0	1	2	3
Feeding Area	0.0%	7.8%	61.7%	30.6%
Feeder Hose	0.5%	4.3%	49.5%	45.7%
Mixer Hose	1.1%	40.9%	35.0%	23.1%
Mixer	0.0%	12.6%	57.7%	29.7%
Hopper	0.0%	6.0%	60.7%	33.3%
Outside of Feeder	0.0%	8.6%	37.6%	53.8%
Area Surrounding Feeder	0.5%	16.7%	28.5%	54.3%
Milk Replacer Handling	0.0%	5.3%	40.4%	54.4%
Pasteurizer	0.0%	0.0%	94.2%	5.8%
Waste Milk Tank	0.0%	0.0%	92.3%	7.7%
<b>Facility item</b>				
Bedding	1.1%	9.7%	55.1%	33.5%
Calf Starter	2.7%	3.8%	31.4%	61.6%
Waterers	3.8%	12.6%	55.7%	27.3%

<sup>1</sup>Score of 0 to 3, with 3 being most ideal; one score per item per visit

Farm	Number of feeders	Feeder	Feed type	Feeder software
VA-1	1	Lely Calm	Milk Replacer	Both
VA-2	1	Lely Calm Combi	Milk Replacer	Both
VA-3	1	DeLaval CF 1000	Milk Replacer	KalbManager
VA-4	1	Lely Calm	Milk Replacer	Institute
VA-5	2	Lely Calm	Milk Replacer	Both
VA-6	1	DeLaval CF 1000	Milk Replacer	Both
MN-1	1	DeLaval CF 1000	Milk Replacer	KalbManager
MN-2	2	DeLaval CF 1000	Milk Replacer	Both
MN-3	2	Lely Calm Combi	Pasteurized Waste Milk	Both
MN-4	2	Lely Calm Combi	Pasteurized Waste Milk	Both

Farm	ADG (kg)	ADHG <sup>2</sup> (cm)
VA-1	0.76	0.18
VA-2	0.88	0.16
VA-3	0.66	0.19
VA-4	0.63	0.18
VA-5	0.64	0.18
VA-6 <sup>3</sup>	0.82	0.21
MN-1	0.57	
MN-2	0.85	
MN-3	0.82	
MN-4	0.90	

<sup>1</sup>Estimated by:

$[(\text{mean calf weight within 5 d of weaning}) - (\text{mean calf weight within 5 d of feeder enrollment})] / \text{feeding plan length}$

<sup>2</sup>Average daily growth in hip height; only measured on VA farms

<sup>3</sup>ADG and ADHG calculated for individual calves routinely; means here represent means for calves enrolled in study

Farm	Mean	SD	n
VA-1	6.16	0.70	14
VA-2	6.07	1.16	16
VA-3	6.72	1.01	19
VA-4	5.82	1.22	13
VA-5	5.28	0.88	39
VA-6	5.46	0.53	20
MN-1	5.62	0.71	81
MN-2	5.43	1.04	37
MN-3	5.66	0.64	149
MN-4	6.13	0.60	94

Farm	% Calves treated	% Calves died	n
VA-1	91.2	0.6	92
VA-2	43.6	3.6	56
VA-3	71.7	1.1	94
VA-4	64.6	2.4	83
VA-5	51.4	6.4	174
VA-6	44.8	0.0	88
MN-1	52.5	0.0	139
MN-2	78.8	3.2	311
MN-3	47.2	1.8	217
MN-4	71.6	0.7	148

<sup>1</sup>Derived from treatment records collected during farm visits

Table 7. Cleaning cycle management by farm

Farm	CC <sup>1</sup> (times/wk) <sup>2</sup>	MCHE <sup>3</sup> (times/d)	Scheduled MCHE times			
			Time 1	Time 2	Time 3	Time 4
VA-1	5	2	15:05	22:00	N/A	N/A
VA-2	4	4	03:00	09:00	15:00	21:00
VA-3	5	3	04:00	14:00	22:00	N/A
VA-4	3	3	07:00	14:00	22:00	N/A
VA-5	4	2	07:00	22:00	N/A	N/A
VA-6	7	2	01:00	17:00	N/A	N/A
MN-1	1	3	07:00	15:00	21:00	N/A
MN-2	7	4	05:00	12:00	16:00	23:00
MN-3	2	2	00:00	12:00	N/A	N/A
MN-4	2	3	01:00	11:00	20:00	N/A

<sup>1</sup>Circuit cleaning: manually initiated cleaning cycle consisting of pre-wash rinse, wash with recirculating heated water and alkaline or acid detergent, and post-wash rinse

<sup>2</sup>If applicable, determined from data derived from Institute software; calculated by: (Total CC recorded)/(# of wk recorded). If Institute was not available, CC frequency was determined by CC records kept by producer or initial survey conducted at start of study

<sup>3</sup>Mixer/heat exchanger cleaning: automatically initiated cleaning cycle consisting of pre-wash rinse, short duration non-recirculating wash, and post-wash rinse; HE only available in Lely Calm Combi feeders

Table 8. Feeder sanitation management by farm

Farm	Detergent (mL/L water)	CC <sup>1</sup> detergent	Additional chlorine bleach use	Hose type
VA-1	10	Peroxide acid mix	Yes	Silicone
VA-2	8	Chlorinated alkaline detergent	No	Silicone
VA-3	10	Chlorinated alkaline detergent	No	Plastic
VA-4	5	Alkaline detergent	Yes	Vinyl
VA-5	10	Chlorinated alkaline detergent	No	Silicone
VA-6	25	Chlorinated alkaline detergent	No	Vinyl
MN-1	15	Chlorinated alkaline detergent	No	Vinyl
MN-2	12	Chlorinated alkaline detergent	No	Vinyl
MN-3	16	Acid detergent	Yes	Plastic
MN-4	15-20	Acid detergent/ Chlorinated alkaline detergent	Yes	Plastic

<sup>1</sup>Circuit cleaning: manually initiated cleaning cycle consisting of pre-wash rinse, wash with recirculating heated water and alkaline or acid detergent, and post-wash rinse



Table 9. Daily feeding behavior from automated feeder software<sup>1</sup>

	Mean	Min	Max	Days recorded <sup>2</sup>
Kalb Manager summary by feeder <sup>2</sup>				
MMR consumed (L) <sup>3</sup>	137	0	776	2105
Calves enrolled	26	1	100	2110
Rewarded visits	95	0	406	2056
Unrewarded visits	182	0	851	2056
Institute summary by feeder <sup>4</sup>				
MMR consumed (L)	150	3	438	1547
Calves enrolled	26	1	71	1547
Rewarded visits	105	2	705	1547
Unrewarded visits	206	1	797	1547

<sup>1</sup>Days when some number (including 0) was recorded here

<sup>2</sup>Derived from all available Kalb Manager data for each farm with access. It covers the dates for each farm from the start to the end of visits. This is not limited to only sample days.

<sup>3</sup>Volume of milk or milk replacer consumed daily

<sup>4</sup>This data is derived from all available Institute data for each farm with access. It covers the dates for each farm from the start to the end of visits. This is not limited to only sample days.

Table 10. Within machine correlation between KM and IS records<sup>1</sup>

Daily record	Correlation
Milk or milk replacer consumed (L) (n = 83)	0.80
Calves enrolled (n = 85)	0.56
Rewarded visits (n = 87)	0.85
Unrewarded visits (n= 87)	0.73

<sup>1</sup>Compares records of Kalb Manager (KM) and Institute (IS) software programs on days samples were collected during biweekly farm visits

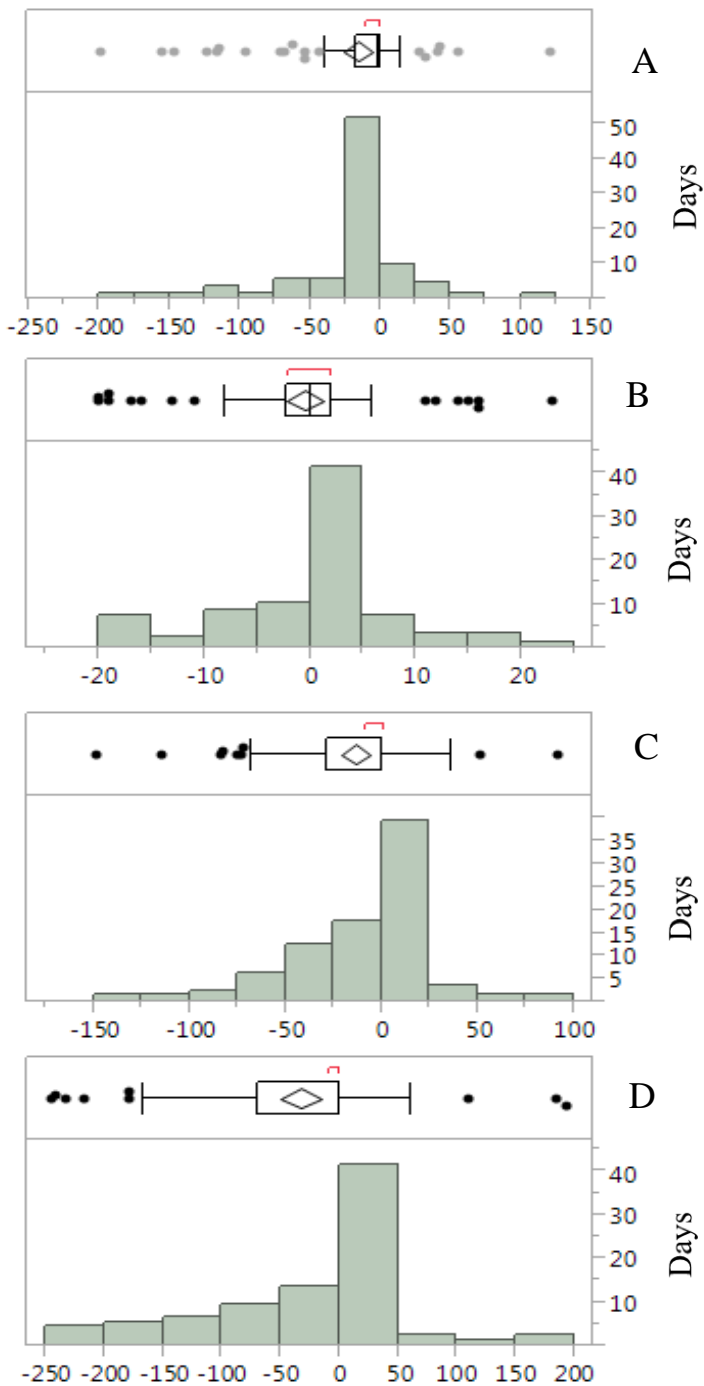


Figure 1. Distribution of differences between Kalb Manager and Institute software data. Differences between data from the two programs, determined by subtracting Kalb Manager value by Institute value on the same day. (A) milk or milk replacer L/d (B) number of enrolled calves (C) rewarded visits (D) unrewarded visits

## CHAPTER 2. FACTORS ASSOCIATED WITH BACTERIAL COUNTS IN AUTOMATED CALF FEEDERS

### ABSTRACT

The objective was to identify factors associated with bacterial counts in automated calf feeders. Six dairy calf operations in Virginia and 4 in Minnesota employing 1 to 2 sophisticated automated calf feeders were visited biweekly for 26 to 28 wk. Observations on facilities and milk or milk replacer (MMR) samples were collected at each visit. Additional milk replacer (MR) samples were collected for 4 wk pre- and post-circuit cleaning. Samples of MMR were plated on Aerobic Plate Count (APC) and Coliform Count (CCP) Petrifilms. Least squares mean APC and CCP were 5.26 and 3.01 log<sub>10</sub> cfu/ml for Virginia and 3.80 and 0.61 log<sub>10</sub> cfu/ml for Minnesota farms. Circuit cleaning (CC) caused 13 and 16% log reduction in APC and CCP. However, for each additional CC/wk, APC and CCP increased by 0.13 and 0.23 log<sub>10</sub> cfu/ml during biweekly farm visits ( $P < 0.001$ ). Each additional mixer/heat exchanger cleaning (MCHE) per d decreased APC and CCP by 1.17 and 1.03 log<sub>10</sub> cfu/ml during biweekly farm visits ( $P < 0.0001$ ), and each hour passed since the last MCHE increased APC and CCP by 0.12 and 0.10 log<sub>10</sub> cfu/ml ( $P < 0.0001$ ) in samples collected before CC. Use of chlorine bleach during cleaning reduced APC and CCP by 0.59 and 1.15 log<sub>10</sub> cfu/ml in samples from biweekly farm visits ( $P < 0.0001$ ), and use of silicone feeder hoses increased APC and CCP by 0.66 and 1.53 log<sub>10</sub> cfu/ml in samples collected before CC ( $P < 0.01$ ). A quadratic effect of MMR liters delivered/d was observed; liters delivered  $> 147$  L decreased APC. Producers using automated feeders should not rely on CC to maintain low bacterial counts in MMR delivered to calves. Performing at least 4 MCHE/d is perhaps the most effective and simple sanitation practice to reduce bacteria.

## **INTRODUCTION**

Young dairy calves are susceptible to disease, and their risk is increased when housed in groups, as with automated feeding systems. There is a common perception that ingestion of high bacterial counts increases the risk of disease in preweaned calves. Despite their importance, proper management protocols regarding sanitation are often unclear to producers using automated feeders. Effectiveness of feeder sanitation management practices has not been studied in field situations, which may explain why recommendations are limited and variable. Farms often differ in their protocols for cleaning frequency, cleaning agents, and hose type. This study serves to identify management factors associated with levels of bacteria in order to provide improved recommendations for automated feeder sanitation.

## **REVIEW OF LITERATURE**

### ***LIQUID FEED INTENDED FOR CALVES***

Calves are fed milk replacer, saleable or nonsaleable milk, or a combination of both with an automated feeder. Nonsaleable milk may include milk from cows treated with antibiotics for mastitis or other bacterial infections, or transition milk from cows that have calved fewer than seven milkings prior to collection (Davis and Drackley, 1998). In the United States, 70.2%, 29.4%, and 33.4% of dairy operations fed preweaned calves milk replacer, saleable milk, and nonsaleable milk, respectively (USDA, 2007).

The nutrient content and warm temperature of milk or milk replacer (MMR) provides an excellent media for bacterial growth. Previous research has focused on contamination of liquid feed prior to feeding preparation, and not on contamination that occurs from mixing/feeding equipment prior to ingestion by the calf.

### ***WHY CONTAMINATION IS A CONCERN***

Research on the direct effects of bacteria levels in MMR on calf health and performance is limited. Jamaluddin et al. (1996) compared health and growth performance of calves fed either pasteurized colostrum and waste milk or raw colostrum and waste milk. Those fed the pasteurized feed had a later onset, shorter duration and less severe diarrhea than those fed unpasteurized milk, suggesting that a lower level of bacteria is beneficial to calf gastrointestinal health (Jamaluddin et al., 1996). Implications for the persistent benefits of reduced bacteria were demonstrated at 180 d of age, when calves fed pasteurized milk were an average of 3.7 kg heavier than calves fed raw milk (Jamaluddin et al., 1996). However, it is worth noting that no analysis was done to compare the levels of bacteria in either treatment feed.

Despite limited research, it is logical to strive for low levels of bacteria in MMR. According to McGuirk (2003), contaminated MMR is a key source of infection of both enteric and respiratory diseases. Cho and Yoon (2014) suggested that exposure to pathogens in a calf's environment is the major cause of diarrhea. Disregarding the presence of bacteria in MMR, calves fed on automated feeders are at a higher risk of pathogen exposure than calves housed individually, since they are grouped with other calves. In group housing systems, it is easy for older calves to transmit pathogens to younger calves through direct contact. Thus, preventing ingestion of bacteria through liquid feed should be a priority in automated feeder systems.

### ***CAUSATIVE AGENTS OF CALFHOOD DIARRHEA***

*Salmonella* spp., *Escherichia coli*, and *Clostridium perfringens* are known to cause scours in calves, and levels of these bacteria should be monitored in MMR (Cho and Yoon, 2014; McGuirk, 2014). *Salmonella* can affect young and mature cattle, but it commonly infects calves under three weeks of age (Cho and Yoon, 2014). The most prevalent serotype causing salmonellosis in US dairy calves is *S. typhimurium*, which invades the intestinal mucosa, causing acute diarrhea (Cho and Yoon, 2014). Enterotoxigenic *E. coli* (ETEC) that produces the K99 adhesion factor is the *E. coli* pathogroup most associated with calf scours (Cho and Yoon, 2014). Newborn calves under a week of age are most susceptible to ETEC-associated scours (Cho and Yoon, 2014). *Clostridium perfringens* type C is typically associated with necrotic enteritis in newborn calves. It produces alpha ( $\alpha$ ) and beta ( $\beta$ ) toxins, which promote cell lysis and mucosal necrosis (Petit et al., 1999).

Viral diarrhea is also common in young calves. Bovine rotavirus is a major infectious agent leading to diarrhea in calves between 1 and 2 weeks of age. If established in the host prior to *E. coli* infection, rotavirus also enhances the pathogenicity of *E. coli* (Roy, 1980). Bovine

coronavirus also causes diarrhea in 1- to 2-week-old calves. Other viruses known to cause diarrhea in young calves include bovine viral diarrhea virus, torovirus, norovirus, and nebovirus (Cho and Yoon, 2014).

### ***CONTAMINATION OF MILK OR MILK REPLACER***

In contrast to milk replacer, milk comes in contact with multiple sources of contamination before flowing through an automated feeder. Prior to any handling, milk is subject to contamination directly from the mammary gland. Considering that a high proportion of nonsaleable milk comes from mastitic cows, there is potential for an elevated level of pathogens to be present in nonsaleable milk intended for calves. *Mycobacterium avium subspecies paratuberculosis* (agent responsible for Johne's disease), *Salmonella* spp., *Mycoplasma* spp., *Listeria monocytogenes*, *Campylobacter* spp., *Mycobacterium bovis*, *Escherichia coli*, are pathogens that may be transmitted in the milk (Godden et al., 2003). Pathogens such as *Listeria monocytogenes*, *Campylobacter* spp., *Pseudomonas* spp., *Bacillus* spp., *Escherichia coli*, *Enterobacter* spp., and *Klebsiella* may contaminate milk from the teat surface during milking (Bremer et al., 2009).

In addition to bacteria transmitted from the mammary gland and teats, bacteria present on the surfaces of milking equipment, buckets, storage tanks, pasteurization equipment, and transportation equipment can contaminate milk. Stewart et al. (2005) identified collection buckets as a source of contamination, demonstrating a mean increase of 3.55 and 3.81 log<sub>10</sub> cfu/ml total plate count and coliform count in colostrum from udder to bucket ( $P < 0.05$ ).

Obviously, storage and pasteurization methods have an effect on the level of bacteria present in milk as well. To ensure safe levels of bacteria for human consumption, the USDA requires saleable bulk tank milk to be cooled to a maximum of 7 °C within two hours following

milking and stored at no higher than that temperature until processed (FDA, 2011). Following pasteurization, milk must be cooled immediately to a maximum of 7 °C again (FDA, 2011). Storing milk intended for calves at higher temperatures before or after pasteurization may encourage bacterial growth.

According to the USDA (2007), only 6.7% of the dairy operations that feed saleable or nonsaleable milk to calves pasteurize it prior to feeding. Nonetheless, pasteurization of milk has shown to be effective in reducing levels of bacteria. Elizondo-Salazar et al. (2010) observed the effectiveness of on-farm batch and high-temperature, short time (HTST) pasteurizers in reducing bacteria levels in nonsaleable milk. Pasteurization resulted in a mean % log reduction of greater than 50% bacteria for all types of bacteria studied (standard plate count, environmental streptococci, coagulase-negative staphylococci, coliforms, and noncoliforms) ( $P < 0.001$ ). With this reduction of bacteria, mean levels met industry-recommended goals of  $< 20,000$  cfu/ml standard plate count (Godden et al., 2005; FDA, 2011),  $< 100$  coliform count (FDA, 2011), and  $< 5,000$  environmental streptococci, coagulase-negative staphylococci, and noncoliforms (McGuirk, 2003). The researchers concluded that successful pasteurization programs should achieve a 50% log reduction in bacteria.

Although pasteurization is effective in reducing bacteria levels in milk fed to calves, it is critical to avoid recontamination of pasteurized milk. In the same pasteurization study mentioned above, Elizondo-Salazar et al. (2010) collected pasteurized milk from random feeding buckets. Bacteria levels from buckets were significantly higher than those found in the milk directly after pasteurization, exceeding the safety levels recommended by Godden et al. (2005), FDA (2011), and McGuirk (2003) ( $P < 0.001$ ). Improper postharvest management of milk can lead to calves ingesting high bacteria loads.



Due to the aforementioned sources of contamination in milk or colostrum, it is expected that these types of feed would contain a higher level of bacteria when fed to the calf than milk replacer. Milk replacer encounters fewer sources of contamination and is typically fed to calves immediately with minimal storage time. Assuming that the water source used to reconstitute milk replacer is uncontaminated, bacteria levels should be very low in the final product. In an observational study by Selim and Cullor (1997), it was found that the viable bacterial counts in milk replacer were nearly 3 and 1 log<sub>10</sub> cfu/ml lower than those in hospital milk and colostrum ( $P < 0.05$ ). Minimal research has been conducted to quantify normal or safe levels of bacteria in reconstituted milk replacer. The Pasteurized Milk Ordinance has established a limit of 20,000 cfu/ml total bacteria for pasteurized milk for human consumption, and may serve as the basis for goals in liquid feed for calves. Researchers have set more specific goals for maximum levels of bacteria in milk replacer (Table 11).

#### ***CONTAMINATION WITHIN THE AUTOMATED CALF FEEDER***

Few researchers have examined the bacterial contamination of MMR that occurs within automated feeders. As MMR flows through an automated feeder, it comes in contact with a variety of materials. According to the Förster Technik calf feeder operational manual, the following materials are exposed to MMR within this type of machine: brass, silicon carbide, stainless steel, plastic, Viton, vulcanized fiber, rubber, and bronze. To feed a calf milk replacer by automated feeder, the powder is first dispensed from a plastic or stainless steel hopper into a mixer where warm water is also dispensed, and the two ingredients are mixed by a stainless steel blade in the bottom of the mixer. Units feeding milk heat the liquid in a heat exchanger prior to mixing. The feed then flows out of the mixer through a hose, then diverts to one of two feeder hoses. Feeder hoses may be constructed of vinyl, silicone, or plastic, depending on producer

preference. The feed may or may not flow through a metal connection that leads to a rubber artificial teat. The teat is accessed by a calf directly (Figure 2).

When MMR comes in contact with internal surfaces of an automated feeder, it is at risk for bacterial contamination. It can be contaminated by individual cells or by the sloughing or breaking up of biofilms present on such surfaces (Bremer et al., 2009).

Biofilms are microbial communities comprised of cells that are irreversibly attached to a surface, so much so that they cannot be removed by rinsing (Agle, 2007; Cloete et al., 2009). The cells of a biofilm are embedded in a matrix of extracellular polymeric substances, like polysaccharides and proteins (Cloete et al., 2009). Biofilms are usually made up of multiple species of bacteria, commonly including pathogens (Agle, 2007; Cloete et al., 2009). These structures develop as a form of protection for the microorganisms, defending against disinfectants and other cleaning agents (Agle, 2007; Cloete et al., 2009).

The development of a biofilm starts with bacteria attaching to a surface, such as stainless steel. Bacteria then multiply and begin to form the exopolysaccharide matrix that secures them within the biofilm (Cloete et al., 2009). Multiple factors influence biofilm formation on milk processing surfaces. Surfaces exposed to an abundance of nutrients, as in the case with milk, encourage growth of densely packed, thick biofilms, especially if these are in locations difficult to clean (Cloete et al., 2009).

Certain surface properties influence formation of biofilms as well. Surfaces that are rough with cracks and crevices allow for more microbial attachment as compared to smooth, undamaged surfaces (Bremer et al., 2009; Cloete et al., 2009). Roughness prevents effective cleaning of surfaces as well (Bremer et al., 2009; Cloete et al., 2009). The physiochemical nature of surfaces also affects biofilm growth. Materials that are hydrophobic, such as plastic, are more

prone to biofilm growth than those that are hydrophilic, such as glass (Cloete et al., 2009).

Biofilms commonly form on the following surfaces found in food production: stainless steel, Teflon, plastic, rubber, and synthetic rubber (Bremer et al., 2009).

Factors of time and temperature also affect biofilm growth, especially within the dairy processing industry. Several dairy processing procedures involve heat, which is why biofilm development occurs within 8 to 12 h in such environments, whereas biofilm growth may take many days in other types of food production (Bremer et al., 2009). Specifically, mesophilic bacteria raise concerns in dairy production, since they grow best at temperatures between 10 and 45 °C (Bremer et al., 2009). Growth of these bacteria may be a concern in automated calf feeders, as MMR is prepared and fed within this temperature range.

Milk or milk replacer flow rate can play a role in the degree of biofilm formation as well. Increased flow rate has been associated with reduced biofilm growth and substrate consumption by microbes. However, high rates of flow may contribute to increased cell release from biofilms, conceivably causing increased bacteria in the MMR ingested by calves (Bremer et al., 2009). Flow rates of MMR in an automated feeder vary between suckling calves, but are likely lower than the standard velocity of 1.5 m/s seen in milk processing (Bremer et al., 2009). Assuming that a calf can drink from an automated feeder at a rate of 1 L/min with a hose diameter of 1 cm, the milk velocity is 0.21 m/s, which is low enough to encourage substrate consumption and biofilm formation.

### ***CONTROL OF BIOFILMS THROUGH CLEANING PRACTICES***

In dairy manufacturing plants, biofilm control is achieved primarily by cleaning, particularly through clean-in-place (CIP) practices (Bremer et al., 2009). The cleaning of automated calf feeders is performed with CIP practices as well, meaning that the machine is

cleaned without disassembly. Clean-in-place practices are advantageous as compared to manual cleaning of individual parts because they are usually more reliable and consistent, and can improve efficiency of labor, water, and cleaning agents (Bremer et al., 2009).

Cleaning agents used in the food and beverage industries are designed to remove nutrient deposits, and not necessarily biofilms directly (Chmielewski and Frank, 2007). Several mechanisms are involved in the removal of specific nutrients. Cleaners used in the food and beverage industries contain agents that chelate minerals, emulsify and saponify lipids, hydrolyze proteins, and decrease surface tension (Chmielewski and Frank, 2007). In dairy processing, the most commonly used alkaline detergent is sodium hydroxide, which specifically is used to remove organic matter, typically leaving behind a mineral layer (Bremer et al., 2009). Alkaline cleaners also have shown to be effective in the removal of biofilms from food processing surfaces, like stainless steel (Chmielewski and Frank, 2007). Acid detergents are designed to remove the mineral layer left behind by alkaline detergents and make the environmental surfaces more bacteriostatic (Bremer et al., 2009). The correct combination of temperature and detergent concentration are critical for effectiveness of acid detergents (Bremer et al., 2009), however, few detailed recommendations exist for correct use of detergents in automated calf feeders.

As expected, the effectiveness of cleaning agents depends upon several factors. The concentration of the cleaner must be sufficient for nutrient and biofilm removal, however, increasing the concentration does not linearly increase effectiveness, since a saturation point may be achieved (Chmielewski and Frank, 2007). Cleaning temperature is critical to effectiveness of cleaning practices. For most food processing surfaces, it is recommended that the cleaning temperature falls between 40 and 90 °C (Chmielewski and Frank, 2007). In milk processing particularly, temperatures that are too hot cause milk proteins to adhere processing surfaces,

causing a counter effective result (Bremer et al., 2009). Förster Technik automated feeders generally clean at a maximum temperature of 50 °C, with a default setting of 45 °C. Since automated feeder CIP procedures operate at relatively low temperatures, it is conceivable that cells remaining after cleaning are still viable and may continue to contribute to biofilm growth (Bremer et al., 2009).

Since published research on the cleaning of automated feeders is unavailable, recommendations for CIP practices in dairy manufacturing plants are discussed here. Five common steps are typically utilized. Pre-rinsing is performed with ambient temperature water to remove loose particles of dairy products. A heated (70 to 80 °C) alkaline wash follows, with recirculation of the solution being necessary for sufficient contact time. The alkaline solution is then rinsed away with ambient temperature water. Following the second rinse, a heated (55 to 80 °C) acid rinse is used for the purpose of removing any residual alkaline cleaner and mineral deposits. The CIP practice is completed with a post-rinse of water or recirculating sanitizer, which may or may not be heated (Bremer et al., 2009).

For comparison, a circuit cleaning (CC) in a Förster Technik automated feeder involves similar, but fewer steps. Ambient temperature water first rinses the mixer and hoses. This is followed by a heated wash cycle of recirculating alkaline (commonly recommended) or acid detergent. A post-wash rinse with water completes the cleaning procedure. An additional, but less extensive cleaning procedure is typically performed at least twice throughout the day according to automated cleaning settings. For the purposes of this paper, these cleanings are referred to as mixer/heat exchanger cleanings (MCHE). In this procedure, ambient temperature water rinses the mixer and hoses (and heat exchanger if applicable). A non-recirculating wash

step is then performed with or without a cleaning agent. Another rinse with water completes the MCHE (Förster Technik Manual, 2011).

### ***DISEASE TRANSMISSION BETWEEN CALVES VIA FEEDING EQUIPMENT***

A common argument against the use of automated calf feeders is that calves share the same nipple, making it easier to transmit contagious diseases between calves. While some automated feeders are capable of sanitizing the nipple between calves, this is not a common feature on most feeders used in the US. It is recommended that calf feeding equipment should be sanitized between calves, no matter if calves are fed via bottles, buckets, or automated feeder (Broadwater, 2011). However, the USDA (2007) reported that only 24.4% of calf operations feeding calves traditionally cleaned nipples, bottles, and buckets between calves, 58.8% of operations cleaned equipment daily, and at least 9.6% of operations cleaned equipment less frequently.

As with traditional calf feeding practices, frequency of cleaning automated feeding equipment is dependent upon established sanitation management and protocols. Although not within the scope of the current study, the risk of disease transmission from feeding equipment may be just as high from nipples, bottles, and buckets as nipples on automated feeders when neither sets of equipment are sanitized between calves.

### ***MONITORING BACTERIA WITH PETRIFILMS***

Petrifilms are dry, rehydratable culture medium systems made up of a plastic film coated with sample-ready media, and another plastic film to cover the media. Both the Petrifilm Aerobic Plate Count and Coliform Count methods are fit for enumerating bacteria in samples of all dairy products (Wehr and Frank, 2004). In comparison to standard methods of plating samples, Petrifilms take up considerably less space, allowing for more samples to be plated with fewer

resources. Curiale et al. (1989) showed that Aerobic Plate Count and Coliform Count methods were equivalent to or more effective than standard methods of enumerating total aerobic bacteria by standard plate count (SPC) and total coliforms by violet red bile agar (VRBA). Cameron et al. (2013) recognized the Petrifilm Aerobic Plate Count as an acceptable on-farm method of quickly identifying cows with intramammary infections through plating milk samples, when compared to standard methods. This suggests that Petrifilm methods may not only be effective for monitoring bacterial counts in MMR in a laboratory setting, but may be feasible on-farm so that producers may regularly test for contamination.

## **MATERIALS AND METHODS**

### ***SAMPLE COLLECTION***

Sampling period timelines are illustrated in Figure 3. During VA farm visits, duplicate milk replacer samples were collected aseptically into 15 ml tubes at the end of the feeder hose from each feeder in use. If a calf was drinking from the feeder at the time of sample collection, the flow was interrupted to collect the sample from that portion. If a milk replacer portion had to be mixed to create a sample, the liquid was expelled for at least 3 s prior to collection to flush out any residual water present in the feeder hose. Samples were transported on ice until arrival at the Virginia Tech Mastitis & Immunology Laboratory where they were frozen at -20 °C. During MN farm visits, samples were collected in the same manner using 50 ml tubes. Single samples from MN were shipped overnight to the Virginia Tech Mastitis & Immunology Laboratory on ice in 2 shipments, checked to ensure they had not thawed, and were immediately stored at -20 °C.

### ***PRE- AND POST-CIRCUIT CLEANING SAMPLING***

For a 4-wk period in July and August, 2014, VA producers were instructed to collect duplicate milk replacer samples with the same method described above. Sample collection was to be performed daily immediately before and after a circuit cleaning (CC), and once on days that a CC was not performed. Farm staff was instructed to store samples in racks in on-farm standard household freezers immediately after collection. Two weeks after the start of the sampling period, research personnel collected the frozen samples during routine farm visits, transported them on ice, and stored them at -20 °C upon arrival to the laboratory. Samples were checked visually to ensure that they remained frozen during transportation. At the next farm visit, samples were collected, transported, and stored in the same manner to complete the sampling period.



## ***BACTERIOLOGY***

Milk and milk replacer samples were analyzed for bacterial numbers using the Petrifilm System (3M, St. Paul, MN). Samples were thawed at room temperature. To perform tenfold serial dilutions, thawed samples were inverted at least 5 times or until solids appeared to be no longer separated, and 3 ml were pipetted into an empty 5 ml plastic tube. This sample tube was inverted at least 5 times and 300  $\mu$ l were pipetted into 2700  $\mu$ l of sterile phosphate buffered saline (PBS) in a 5 ml plastic tube. Immediately, this tube was inverted at least twice, and 300  $\mu$ l of it was pipetted into 2700  $\mu$ l of PBS. For the first few laboratory sessions, this procedure continued until a dilution of 1:100,000 was achieved. One ml of each dilution was plated on 1 Aerobic Plate Count (APC) Petrifilm and 1 ml was plated on 1 Coliform Count (CCP) Petrifilm (3M, St. Paul, MN). At 37°C, APC Petrifilms were incubated for  $48 \pm 3$  h and CCP Petrifilms for  $24 \pm 2$  h. Following plating, samples were returned to the freezer (-20 °C).

Following incubation, the most suitable dilution (within the range of 20 to 250 cfu) for each sample, i.e. the Petrifilm with the fewest colony forming units, was used to estimate bacterial numbers. Single red colonies regardless of size were considered 1 cfu. Gas colonies on the CCP Petrifilms were considered confirmed coliforms. If samples from a VA farm were uncountable because of liquification on the APC Petrifilms, extremely large gas bubbles on the CCP Petrifilms, or too numerous to count (TNTC) colonies, the duplicates were plated to a higher dilution. Because there was no access to duplicate samples from MN farms, MN sample dilutions that were uncountable were re-thawed and re-plated.

After multiple samples from each farm were plated and bacterial counts were evaluated, adjustments were made to the maximum dilution plated on APC or CCP Petrifilms. For instance, if multiple samples from a farm were within the countable range of bacteria at a dilution of

1:100,000, dilutions for that farm were increased to 1:1,000,000 (which was the maximum dilution reached throughout the study). Conversely, if no bacteria were detected on the 1:100,000 Petrifilm, and few were detected on the preceding dilution, samples from that farm were decreased to a dilution of 1:10,000. Similarly, the range of bacterial counts were analyzed to determine which minimum and maximum dilutions should be plated on APC and CCP Petrifilms for subsequent plating sessions.

## ***STATISTICAL ANALYSES***

### ***Study Variables***

Prior to analysis, variables were characterized as either between- or within-machine variables. With few exceptions, between-machine variables remained the same on a machine throughout the study, indicating variation was among farms. These variables included circuit cleanings per wk (CC/wk), mixer/heat exchanger cleanings per d (MCHE/d), ml detergent per L water (DET), feeder hose type, and additional use of chlorine bleach (CB). Information on CC/wk was determined by survey responses and validated with service record from Institute software (IS) if applicable. Information on MCHE/d and DET was retrieved from machine cleaning settings viewed during farm visits. Feeder hose type was plastic, silicone, or vinyl, and was determined by survey responses and researcher observations. Use of CB in cleaning was recorded in surveys.

Within-machine variables varied for each machine according to the time of sampling or the day, indicating variation primarily on-farm. Days since last CC (DLCC) and hours since last MCHE (HLMC) indicated the days or hours passed between the last CC or MCHE and the time of the sample collection. Records on CC times were retrieved from the service record in IS, except for VA-3, where CC times were recorded consistently by hand. If available, records on

MCHE times were also retrieved from the service record in IS, otherwise the last scheduled MCHC according to machine settings was used to determine HLMC. Liters of milk or milk replacer consumed on the date the sample was taken (MMR L/d) were retrieved from KM and the IS feeder record. Since data did not always match between the two software programs, the maximum MMR L/d between KM and IS was used with the assumption that it was more likely for the software to undervalue than overvalue milk or milk replacer volume. Number of calves enrolled, rewarded visits (RV), and unrewarded visits (URV) were also considered for inclusion in the statistical models. However, RV and URV were not used because they were not expected to be independent of one another or MMR L/d, and the KM and IS records of these data were less highly correlated than those of the MMR L/d data. Pre-CC APC and CCP counts were considered as variables for the log reduction of APC (LRAPC) and log reduction of CCP (LRCCP) models. However, regressions showed that LRAPC and LRCCP were highly dependent on pre-CC APC and CCP, masking the effects of other variables on bacterial count, and thus were excluded from the models and interpreted separately.

### ***Biweekly Farm Visits***

All bacterial data were measured in cfu/ml and were logarithmically transformed ( $\log_{10}$ ) since raw counts were not normally distributed.

The biweekly farm visit (BFV) data set was reduced to represent only 1 sample collected from each machine every other week throughout the entire study period. To achieve this, MN sampling days representing every other week were removed from the data set. Differences in APC and CCP between states and farms were identified by ANOVA in JMP with the random effect of farm(state). Least squares means (LSM) and standard errors (SE) were derived from the same analysis. To test the effect of between-machine variables on  $\log_{10}$  aerobic plate count

(APC) and  $\log_{10}$  coliform count (CCP), a stepwise regression in JMP was run for APC and CCP. All variables were included in the model and were removed at  $P > 0.10$ , and reentered at  $P \leq 0.10$ . To identify any variables that had a very large effect on the outcome variables, changes in  $R^2$  values were observed as variables entered the model. However, no individual variables had disproportionately large effects on  $R^2$  (adding  $> 0.25$  to  $R^2$ ). Residual plots were checked for normality using PROC REG in SAS.

Days since last circuit cleaning (DLCC) was not included in the BFV model since 3 of the 14 machines did not have CC time records, and a large number of MN farms had few CC time records. To test the effect of the remaining within-machine variables on APC and CCP, SAS GLIMMIX was run with machine(farm) as a random effect. Two-way interactions and squared terms were included in the model initially. Any interaction or squared term was removed at  $P > 0.10$ . Residual plots were checked for normality.

To test the ability to visually assess automated feeder cleanliness, SAS GLIMMIX was run as described above with scores (0 to 3) recorded during farm visits on machine feeder hoses, mixer hoses, mixers, and hoppers included in the model.

### ***Pre-/Post-Circuit Cleaning Sampling***

Although VA farms were instructed to collect samples daily during the 4 wk pre/post-CC sampling period (PPCC), several farms skipped sampling days. The two farms with the fewest samples had 7 d of pre/post-CC samples. Thus, the data were reduced for the other farms to represent pre/post-CC data approximately every 4 d for each machine within PPCC.

To test the effect of between-machine variables on pre-CC APC and CCP, a stepwise regression in JMP was run for APC and CCP as described above. Since DLCC data were available for all but one sample, this variable was included in the pre-CC model. To test the

effect of within-machine variables on pre-CC APC and CCP, SAS GLIMMIX was run as described above.

### ***Log Reduction of Bacteria***

Log<sub>10</sub> reduction (LR) achieved by CC was determined for the PPCC data:

$$LR = \log_{10} \left( \text{pre CC bacteria} \frac{\text{cfu}}{\text{ml}} \right) - \log_{10} \left( \text{post CC bacteria} \frac{\text{cfu}}{\text{ml}} \right)$$

The same formula was used to calculate both LRAPC and LRCCP. Alternatively, LR could be calculated as log<sub>10</sub>(ratio of pre-CC to post-CC cfu/ml). To test the effectiveness of CC, pre-CC and post-CC bacteria were compared by ANOVA in JMP with the random effect of machine(farm). Simple linear regressions were estimated to evaluate the dependency of LR on pre-CC bacterial counts.

To test the effect of between-machine variables on LRAPC and LRCCP, a stepwise regression in JMP was run for APC and CCP as described above. To test the effect of within-machine variables on LRAPC and LRCCP, SAS GLIMMIX was run as described above.

## **RESULTS AND DISCUSSION**

### ***BACTERIAL COUNTS***

Least squares mean APC for MN and VA during biweekly farm visits were 3.80 and 5.26  $\log_{10}$  cfu/ml, or 6,310 and 181,970 cfu/ml ( $P = 0.0037$ ). Coliform counts were 0.61 and 3.01  $\log_{10}$  cfu/ml, or 4 and 1,023 cfu/ml for MN and VA ( $P = 0.0012$ ; Figure 4). Differences between states were likely due to sanitation management rather than climate differences, as temperature was found to have no relationship to bacterial counts. Management of cleaning cycles, cleaning agents, and hose type were not related to state. However, MN farms generally had larger calf operations than VA farms and may have carried out cleaning protocols more routinely. LeBlanc et al. (2006) suggested that larger farms may be more successful in implementing management protocols due to better training and labor management.

Least squares means for individual farms ranged from 3.36 to 5.68  $\log_{10}$ , or 2,291 to 478,630 cfu/ml for APC and 0.12 to 3.66  $\log_{10}$ , or 1 to 4,571 cfu/ml for CCP (Figure 5). Goals established by Godden et al. (2005) and FDA (2011) and adapted by Elizondo-Salazar et al. (2010) for bacteria levels in pasteurized waste milk are  $< 20,000$  and  $< 100$  cfu/ml for standard plate count and coliform count. Three farms met this goal for LSM APC and 5 farms met this goal for LSM CCP. McGuirk (2008) suggested a more stringent goal of  $< 10,000$  and 0 cfu/ml for total bacteria and fecal coliform count. However, no research has yet shown a relationship to calf disease at either of these goals.

### ***SANITATION MANAGEMENT***

#### ***Machine Cleaning Cycles***

Tables 12-17 show the effects of management variables on bacteria and log reduction of bacteria achieved by circuit cleaning (CC). Mixer/heat exchanger cleanings were effective in

reducing bacterial counts in milk or milk replacer (MMR) samples. From the biweekly farm visit study (BFV), 1 additional MCHE/d decreased APC by 1.17 log<sub>10</sub> cfu/ml ( $P < 0.0001$ ; Table 12). The pre-/post-circuit cleaning study (PPCC) showed that if hours since last MCHE (HLMC) increased by 1 h, pre-CC APC increased by 0.12 log<sub>10</sub> cfu/ml ( $P = 0.0022$ ), and pre-CC CCP increased by 0.10 log<sub>10</sub> cfu/ml ( $P < 0.0001$ ; Table 15). However, there was a contrasting tendency for machines with more frequent MCHE/d in PPCC to have higher pre-CC CCP counts ( $P = 0.0613$ ), showing that one additional MCHE/d would increase CCP by 0.41 log<sub>10</sub> cfu/ml (Table 14). To investigate this discrepancy, a stepwise regression was run to test the between-machine variables during biweekly farm visits on VA farms only. Results from this analysis were in agreement with the stepwise results from BFV, showing that each additional MCHE/d decreased APC by 0.60 log<sub>10</sub> cfu/ml ( $P < 0.0001$ ). Thus, the difference in sampling period length and sampling frequency may have caused the conflicting results between BFV and PPCC data. The effects of temperature in the barn or autofeeder room on bacterial counts were examined initially by regression, with no indication of a relationship, so seasonal temperature changes likely did not have an effect. Biweekly farm visits spanned a longer period of time and consisted of more random sample collection than the PPCC sampling period. Therefore, the results from BFV may be more representative of the effects of management practices on bacteria in automated feeders.

Least squares mean post-CC APC and CCP were 0.70 and 0.52 log<sub>10</sub> cfu/ml lower than LSM pre-CC APC and CCP ( $P < 0.0001$ ;  $P = 0.0043$ ). Log reduction (LR) is a ratio of pre-CC to post-CC cfu/ml. The average pre-CC:post-CC ratios were 5.01 and 3.31 for APC (186,209:38,019 cfu/ml) and CCP (1,862:562 cfu/ml). Greater pre-CC bacterial counts resulted in greater LR of bacteria. In other words, the higher the bacterial counts prior to circuit cleaning

(CC), the greater the ratio of pre-CC to post-CC bacteria. For each  $\log_{10}$  cfu/ml increase in pre-CC APC, log reduction of APC (LRAPC) increased by 0.56 ( $P = 0.0003$ ;  $R^2 = 0.24$ ). For each  $\log_{10}$  cfu/ml increase in pre-CC CCP, after reaching a pre-CC CCP of about 1.08  $\log_{10}$  cfu/ml, average log reduction of CCP (LRCCP) increased by 0.12 ( $P = 0.0297$ ;  $R^2 = 0.37$ ).

Due to this relationship, increased LR of bacteria may imply a high concentration of bacteria prior to CC, supporting the negative effect of mixer/heat exchanger cleaning (MCHE) on bacterial counts. An increase in hours since last MCHE (HLMC) by 1 h increased LRCCP by 0.15, increasing the average pre-CC:post-CC ratio to 4.68 ( $P = 0.0018$ ; Table 17). This suggests that pre-CC CCP was high as a result of bacterial growth as time passed since the last MCHE. With this theory in mind, it appears that a similar relationship exists for LRAPC, although a quadratic effect of HLMC on LR was observed. For example, at 1, 3, 5, or 7 h since the last MCHE, LRAPC decreased by 0.15, 0.39, 0.55, and 0.63 resulting in average pre-CC:post-CC ratios of 3.55, 2.04, 1.41, and 1.17 (Figure 7). When more than 16 h passed since the last MCHE, LRAPC increased for each 1 h increase in HLMC ( $P = 0.0006$ ). In other words, if the last MCHE occurred fewer than 16 h prior to the CC, LRAPC decreased as HLMC increased. If 16 h or more passed since the last MCHE, LRAPC was increased as more time passed before the circuit cleaning (CC), likely because more bacteria were present pre-CC. However, all farms on the study performed at least 2 MCHE/d, so it would be uncommon for calves to ingest milk or milk replacer 12 h post-MCHE. In contrast to this idea of pre-CC bacteria increasing with time and causing increased LR, machines with more MCHE/d tended to have higher LRAPC and LRCCP, increasing by 0.30 and 0.41 per additional MCHE/d ( $P = 0.0626$ ;  $P = 0.0868$ ). An alternative explanation could be that increased MCHE frequency could improve the effectiveness of CC.



Log reduction of bacteria was achieved by CC, however, CC may not be effective in helping producers meet the goals suggested by Elizondo-Salazar et al. (2010). While CIP methods in automated feeders are entirely different processes than pasteurizing milk for calves, the end goal is the same: to reduce bacteria to concentrations that are safe for ingestion by the calf. Elizondo-Salazar et al. (2010) suggested using percent log reduction (PctLR) as a measure of effectiveness for waste milk pasteurizers with the suggestion that a 50% PctLR would lower bacteria to safe levels, even with high levels of bacteria in the pre-pasteurized milk. Percent LR was calculated as:

$$\text{PctLR} = \left[ \frac{\left[ \log_{10} \left( \text{pre CC bacteria} \frac{\text{cfu}}{\text{ml}} \right) - \log_{10} \left( \text{post CC bacteria} \frac{\text{cfu}}{\text{ml}} \right) \right]}{\log_{10} \left( \text{pre CC bacteria} \frac{\text{cfu}}{\text{ml}} \right)} \right] * 100$$

Circuit cleaning reduced APC from LSM of 5.27 to 4.58 log<sub>10</sub> cfu/ml, a difference of 148,190 cfu/ml, and CCP from LSM of 3.27 to 2.75 log<sub>10</sub> cfu/ml, a difference of 1,300 cfu/ml (Figure 5). Percent LR achieved by CC for APC and CCP were 13 and 16%. Pre-CC APC and CCP counts were considerably higher than pre-pasteurized counts observed by Elizondo-Salazar et al. (2010) (LSM: 5.27 vs. 4.29; 3.27 vs. 2.14 log<sub>10</sub> cfu/ml). To reach a goal of < 20,000 cfu/ml post-CC APC at a PctLR of 13%, pre-CC APC would need to be < 5 log<sub>10</sub> cfu/ml. To reach a goal of < 100 cfu/ml post-CC CCP at a rate of 16% PctLR, pre-CC CCP would need to be < 2.5 log<sub>10</sub> cfu/ml. Since LSM pre-CC bacterial counts were higher than these, CC alone may not be effective in successfully reducing bacteria to recommended levels for calves.

In comparison to MCHE, CC did not appear to be as effective in lowering cfu for samples taken during farm visits and pre-CC samples. During biweekly farm visits (BFV), increasing the number of CC/wk by 1 increased APC by 0.13 and CCP by 0.23 log<sub>10</sub> cfu/ml (*P* < 0.0001; Table 12). A larger increase of 1.47 log<sub>10</sub> cfu/ml CCP for each additional CC/wk was

observed during the pre-/post-CC study (PPCC) ( $P < 0.0001$ ; Table 14). During PPCC, increased days since last CC (DLCC) also tended to decrease CCP by  $0.27 \log_{10}$  cfu/ml for each day since the last CC ( $P = 0.0653$ ; Table 15). Additionally, LRCCP was increased by  $0.34 \log_{10}$  cfu/ml for each additional CC/wk ( $P = 0.0242$ ; Table 16), supporting the results showing increased pre-CC bacteria. Circuit cleaning reduced the concentration of bacteria immediately, however, CC may not have lasting benefits on maintaining low bacteria concentrations between cleanings.

The MCHE process differs from CC. Therefore it is likely that they could have different effects on bacterial counts immediately after cleaning and in subsequent flushes. Circuit cleaning is the more aggressive cleaning, consisting of a water flush, a wash cycle lasting several minutes, and a post-wash flush. High flow rates have been shown to reduce bacterial adherence and cause a higher rate of cell release (Bremer et al., 2009). Perhaps biofilms located on the inside surfaces of the machine are loosened by CC, leading to sloughing off of bacteria during subsequent milk or milk replacer (MMR) delivery. This could explain why increased CC/wk increased APC and CCP; there may have been more biofilm breakup from increased CC frequency.

On the other hand, MCHE is little more than an automatic flush using detergent, with the only agitation occurring in the mixing bowl. It is possible that bacteria secured in biofilms are undisturbed by MCHE, and only loose or planktonic bacteria are flushed from the machine. It is unlikely that MMR delivery for feeding or sample collection would agitate biofilms enough to break them apart considerably. Therefore the bacteria present in MMR samples from this study may have been planktonic or loosely attached to a biofilm at the time of collection. Assuming MCHE does not thoroughly disrupt biofilms, this may explain why greater MCHE/d decreased APC and CCP and greater HLMC increased APC and CCP in samples. Mixer/heat exchanger cleanings appear to be effective in flushing out planktonic or loosely attached bacteria, thus more

MCHE/d and less time since the last MCHE resulted in lower bacterial counts captured during sample collection.

Nonetheless, CC reduced APC and CCP in this study, so producers should not be discouraged from increasing CC frequency. Circuit cleaning also may be important in preventing biofilm buildup over time, a factor that was not directly examined in this study. Perhaps cleaning times could be adjusted to avoid high bacterial counts in MMR delivered to calves. If CC breaks up biofilms and releases bacteria in MMR during subsequent feedings, those feedings soon after CC should be avoided. Many producers perform CC in the morning right before calves are about to eat. Alternatively, CC could be done after the majority of calves have eaten or at night time to reduce the risk of ingesting bacteria. Jensen (2004) reported the least feeding activity between 22:00 and 03:00, and another mid-morning lull in feeding activity after most calves have visited the feeder. Another strategy may be to follow a CC with a MCHE within the subsequent 1 to 2 h to flush out bacteria loosened by the CC. It also may be helpful to schedule MCHE about 1 h prior to major feeding times to reduce the risk of pathogen ingestion.

Of course, avoiding biofilm breakup begins with prevention of biofilm formation. Unfortunately, each of the machines on this study was in use at the start of sample collection and likely had established biofilms on at least some inside surfaces for the entirety of the study, so biofilm prevention could not be examined. However, machine parts can be replaced to entirely remove biofilms. Feeder hoses are the most frequently replaced parts, and they make up a large proportion of the surface in contact with MMR. Other machine parts are more difficult or expensive to replace, and often remain intact for many months or years. These include the mixer and mixer hose (suction hose), pumps, and various valves. It may be challenging for the producer to more frequently replace these materials and may not be practical or economically justified.

## *Cleaning Agents*

According to the Förster Technik manual, producers should use either an alkaline or acidic cleaning agent that can operate at a temperature of 40 to 50 °C and has no corrosive effect. Concentrations of detergents should be used according to manufacturer label (Ziemerink, personal communication, 2015). The most commonly used detergent on study farms was DeLaval RTD™, a chlorinated alkaline detergent (sodium hypochlorite and sodium hydroxide) designed to clean at 45 °C. Concentrations of this detergent ranged from 10 to 25 ml per L of water. All farms set automated feeders to clean using water at a temperature of 50 °C. A challenge with automated feeders is that they are not designed to easily follow an alkaline wash with an acid rinse, as done for most CIP practices in the dairy industry.

Detergent type was not tested as a variable because most farms used the same type, but concentration was tested. Conflicting bacterial counts were observed between the biweekly farm visit (BFV) samples and pre-/post-circuit cleaning (PPCC) samples regarding the effect of detergent level (DET) on bacterial count. Increasing DET by 1 ml/L of water decreased APC and CCP by 0.07 and 0.10 log<sub>10</sub> cfu/ml ( $P = 0.01235$ ;  $P < 0.0001$ ; Table 12) during the biweekly farm visit study (BFV). However, the pre-/post-CC study (PPCC) showed that machines with increased DET had higher APC; as DET increased by 1 ml/L water, APC increased by 0.06 log<sub>10</sub> cfu/ml ( $P = 0.0016$ ; Table 14). To investigate these discrepancies, a stepwise regression was run to test the between-machine variables during biweekly farm visits on VA farms. Results from this analysis were in agreement with the stepwise results from BFV, showing that DET was associated with lower APC and CCP. Because the PPCC sampling period occurred over a shorter period of time, it may not have captured the effects of sanitation practices as well as the BFV sampling period.

Using the correct detergent concentration is important for nutrient and biofilm removal. The concentration must be sufficient to effectively clean surfaces, but after a saturation point is reached, increasing detergent concentration is not advantageous (Chmielewski and Frank, 2007). Since DET was found to decrease APC and CCP, study farms were likely not using excessive concentrations.

The additional use of chlorine bleach (CB), or household bleach, appears to decrease bacterial counts present in MMR from automated feeders. The use of CB decreased APC and CCP in BFV samples by 0.59 and 1.15  $\log_{10}$  cfu/ml ( $P < 0.0001$ ; Table 12). Similarly, the use of CB reduced CCP by 0.96  $\log_{10}$  cfu/ml in pre-CC PPCC samples ( $P = 0.0061$ ; Table 14). Its effectiveness in cleaning is supported by the tendency of CB use to increase LRAPC by 0.43, causing the average pre-CC:post-CC ratio to increase from 3.31 to 13.49 ( $P = 0.094$ ; Table 16).

Chlorine bleach is a highly accessible disinfectant used often in household cleaning. At a dilution of 5.25% sodium chlorite, CB was shown to effectively remove biofilms and dried-on organisms from medical surfaces, proving more effective than detergents (Merritt et al., 2000). It kills a broad spectrum of bacteria, including pathogens, working by dissociating HOCl<sup>-</sup> (HICPAC, 2008). During both sampling periods of the current study, machines using CB had lower bacterial counts, and thus it is a simple method of reducing bacteria. However, due to the corrosive nature of CB, producers should be aware of the damaging effect it may have on automated feeders.

### ***Hose Type***

Of the 3 feeder hose types used (plastic, PLST; silicone, SIL; vinyl, VIN), SIL resulted in the highest concentrations of bacteria in samples and VIN resulted in the lowest. During PPCC, the use of SIL increased APC and CCP by 0.66 and 1.53  $\log_{10}$  cfu/ml ( $P = 0.0041$ ;  $P < 0.0001$ ;

Table 14). During BFV, the use of VIN decreased CCP by 1.30 log<sub>10</sub> cfu/ml ( $P < 0.0001$ ; Table 12). No effect of hose type was observed on the LR of bacteria.

Numerous surface factors affect the ability of biofilms to form on a material and the ability of a material to be cleaned. The results of this study suggest that VIN is less susceptible to biofilm formation or is more easily cleaned by MCHE or CC than SIL. Silicone tubing has a longer useful life than vinyl tubing (Laboratory and Peristaltic Pump Tubing, 1999), and therefore is less prone to cracks that cause challenges in cleaning. However, since SIL is more expensive and lasts longer than VIN, it is probable that producers using SIL replaced hoses less frequently than producers using VIN. Less frequent hose replacement would lead to increased bacterial growth in SIL. Despite this reasoning, there was no difference between SIL and VIN in the frequency with which producers reported replacing hoses (initial surveys).

### ***Milk/Milk Replacer Volume***

Volume of milk or milk replacer consumed on the day of sampling (MMR L/d) had a quadratic effect on APC counts in the pre-/post-CC study (PPCC). When MMR L/d was less than 147 L/d, increasing MMR increased APC by 0.05 log<sub>10</sub> cfu/ml ( $P = 0.0285$ ; Table 15). When MMR exceeded 147 L/d, increasing MMR decreased APC by 0.0002 log<sub>10</sub> cfu/ml ( $P = 0.0493$ ; Table 15). Figure 8 illustrates the expected APC count at different volumes of MMR L/d, assuming the other significant factor (hours since last MCHE) is average. Assuming that increased LR is a result of high pre-CC bacterial counts, the relationship observed between MMR L/d and LRAPC supports this finding. The regression shows that additional MMR delivered via feeder increased LRAPC until 175 L/d were delivered ( $P = 0.0023$ ), at which point additional MMR delivered through the feeder decreased LRAPC ( $P = 0.0038$ ; Table 17). However, this relationship is not an accurate representation of the effect of MMR L/d on

LRAPC. When examined as an individual variable, LRAPC linearly decrease as MMR increased. It can be concluded that although this model is useful for predicting LR, the variables in this model may be too closely related to interpret the linear and quadratic coefficients clearly.

The point where MMR L/d had no effect on APC (147 L) was very close to the mean estimate of MMR L/d during the entirety of the study period recorded by Institute software (150 L) and Kalb Manager software (137 L). Both the increasing and decreasing effects of MMR L/d on APC are very small. Thus, on average, the volume of MMR should not have a large impact on the bacteria concentration in the machine. However, the predicted APC approaches 0 log<sub>10</sub> cfu/ml at a level of 329 L MMR, which is lower than the maximum recorded for IS (438 L) and KM (776 L) across all farms. At the lowest farm average MMR L/d of nearly 60 L, the predicted APC is 4.48 log<sub>10</sub> cfu/ml. With lower-than-average MMR output per d, there may not be enough liquid flushing the automated feeders to prevent additional bacterial growth, and until 147 L is reached, more MMR delivered through the machine provides more nutrients for bacterial growth. At output rates higher than 147 L/d, it is possible that more MMR flow causes planktonic bacteria to be removed and limits establishment of biofilms. Additionally, the default setting on Förster Technik automated feeders allows calves > 14 d into their feeding plan to consume a portion of water following MMR. With more MMR flowing through the feeder, there is also more water that can flush the system of residual MMR.

While there may be an effect of MMR L/d on the concentration of bacteria present in automated feeders, producers should not be discouraged from operating machines that do not reach the 147 L/d threshold if it is not practical to enroll more calves or increase feeding plans. Instead, they can change the setting on their feeders to provide water to calves of all ages after a

meal to allow more flushing and encourage water intake in calves, or increase their frequency of MCHE.

### *Visual Assessment of Cleanliness*

Automated feeders were scored on cleanliness at each farm visit on a scale of 0 to 3, with 3 being the cleanest score. Although this was a subjective assessment of sanitation, there was a relationship between scores and bacterial counts (Table 18). For each additional point awarded for mixer (M) score cleanliness, CCP decreased by  $0.46 \log_{10}$  cfu/ml ( $P = 0.0076$ ). This suggests that a mixer that appears dirty indicates higher concentrations of CCP in the MMR delivered by the machine.

When scores were below approximately 1.5 and 2 for feeder hose (FH) cleanliness, a 1 point increase was associated with a decrease in APC and CCP of 1.66 and  $2.03 \log_{10}$  cfu/ml ( $P = 0.0088$ ;  $P = 0.0164$ ). However, after those points, a 1 point increase in cleanliness was associated with an increase in APC and CCP of 0.58 and  $0.49 \log_{10}$  cfu/ml ( $P = 0.0012$ ;  $P = 0.0091$ ). This suggests that visual assessment of FH is related to bacteria concentration, but only at low scores of 0 or 1.

Surprisingly, a 1 point increase in mixer hose (MH) cleanliness indicated an increase of  $1.76 \log_{10}$  cfu/ml CCP ( $P = 0.0002$ ). This shows that scoring MH cleanliness gives misleading information for assessing bacteria concentration, likely related to the opaqueness of the silicone MH material. There was an interaction between FH and MH score ( $P = 0.0003$ ). It is possible that researchers' scores of one material influenced their scoring of the other.

While automated feeder visual cleanliness may be related to bacteria concentration, it is important for producers not to rely on this for assessing bacterial growth. A producer that spends time with the same machines each day is unlikely to notice gradual MMR residue buildup,



making it easy to misjudge cleanliness of machines. Therefore, it is highly recommended that protocols for machine sanitation and parts replacement be performed on a regular basis.

### ***CONCLUSION ON SANITATION MANAGEMENT***

Although research has not yet proven that high concentrations of bacteria in milk or milk replacer directly lead to disease in preweaned calves, it is assumed that ingestion of bacteria increases the risk of pathogen ingestion resulting in disease. On average, 3 study farms met the goal of < 20,000 cfu/ml APC and 5 farms met the goal of < 100 cfu/ml CCP adapted from Elizondo-Salazar et al. (2010), demonstrating the achievability of these goals. However several farms, most of which were in VA, were far from meeting these goals.

Producers may reduce risk associated with high bacteria by implementing different management strategies in automated feeder systems. Mixer/heat exchanger cleanings appeared to reduce bacteria, and can easily be performed at least 4x/d. This is one of the easiest practices to implement consistently since mixer/heat exchanger cleanings are initiated automatically and do not require any manual labor. Circuit cleanings should be implemented several times a week, but a producer should not rely on them to effectively reduce high bacterial counts or maintain low bacterial counts over time. Detergent should be used in concentrations recommended by the manufacturer. Chlorine bleach may be used to improve sanitation, but producers should be cautioned on the corrosive effect it has on feeders.

Biofilm development in automated feeders should be investigated more thoroughly. Without further investigation, it is assumed that feeder hoses are likely an area of biofilm buildup and should be replaced frequently to reduce bacterial contamination of milk or milk replacer.

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Source	Total bacterial count	Total coliform count	Total E. coli count	Other gram negative	Strep non-ag	CNS <sup>1</sup>
McGuirk (2003)	< 10,000		0	< 5,000	< 5,000	< 5,000
Wisconsin Vet. Diagnostic Lab (2014)	< 20,000	< 1,000	< 100			

<sup>1</sup>Coagulase-negative staphylococci

Variable <sup>4</sup>	APC <sup>2</sup> (R <sup>2</sup> = 0.53)			CCP <sup>3</sup> (R <sup>2</sup> = 0.47)		
	Estimate	SE	P value	Estimate	SE	P value
Intercept	8.46	0.35		6.09	0.59	
Mixer/heat exchanger cleanings/d	-1.17	0.08	< 0.0001	-1.03	0.14	< 0.0001
Circuit cleanings/wk	0.13	0.03	0.0002	0.23	0.06	< 0.0001
Detergent level (ml/L water)	-0.07	0.01	< 0.0001	-0.10	0.02	< 0.0001
Chlorine bleach use (1 = yes, 0 = no)	-0.59	0.14	< 0.0001	-1.15	0.22	< 0.0001
Silicon feeder hose (1 = yes, 0 = no)			0.4467			0.9834
Vinyl feeder hose (1 = yes, 0 = no)			0.3146	-1.30	0.22	< 0.0001
Plastic feeder hose (1 = yes, 0 = no)			0.6715			0.9834

<sup>1</sup>Samples from MN and VA farms

<sup>2</sup>Aerobic plate count (log<sub>10</sub> cfu/ml)

<sup>3</sup>Coliform count (log<sub>10</sub> cfu/ml)

<sup>4</sup>Variables with no estimates were removed for lack of significance prior to final model

Variable	APC <sup>2</sup>			CCP <sup>3</sup>		
	Estimate	SE	P value	Estimate	SE	P value
Intercept	4.58	0.29		1.49	0.46	
HLMC <sup>4</sup>	0.02	0.01	0.2019	0.01	0.02	0.5245
MMR L/d <sup>5</sup>	-0.001	0.00	0.3297	0.001	0.00	0.5156

<sup>1</sup>Samples from MN and VA farms

<sup>2</sup>Aerobic plate count (log<sub>10</sub> cfu/ml)

<sup>3</sup>Coliform count (log<sub>10</sub> cfu/ml)

<sup>4</sup>Hours since last mixer/heat exchanger cleaning

<sup>5</sup>Milk or milk replacer delivered on day of sample collection



Table 14. Effect of between machine variables on bacterial counts before circuit cleaning<sup>1</sup> (n = 49)

Variable <sup>4</sup>	APC <sup>2</sup> (R <sup>2</sup> = 0.25)			CCP <sup>3</sup> (R <sup>2</sup> = 0.73)		
	Estimate	SE	P value	Estimate	SE	P value
Intercept	4.27	0.29		-3.81	1.08	
Mixer/heat exchanger cleanings/d			0.6669	0.41	0.12	0.0613
Circuit cleanings/wk			0.8128	1.47	0.29	< 0.0001
Detergent level (ml/L water)	0.06	0.18	0.0016	-0.11	0.07	0.0942
Chlorine bleach use (1 = yes, 0 = no)			0.3386	-0.96	0.33	0.0061
Silicone feeder hose (1 = yes, 0 = no)	0.66	0.22	0.0041	1.53	0.29	< 0.0001

<sup>1</sup>Samples collected during 4-wk pre/post circuit cleaning study on VA farms only

<sup>2</sup>Aerobic plate count (log<sub>10</sub> cfu/ml)

<sup>3</sup>Coliform count (log<sub>10</sub> cfu/ml)

<sup>4</sup>Variables with no estimates were removed for lack of significance prior to final model

Table 15. Effect of within machine variables on bacterial counts before circuit cleaning<sup>1</sup> (n = 49)

Variable	APC <sup>2</sup>			CCP <sup>3</sup>		
	Estimate	SE	P value	Estimate	SE	P value
Intercept	1.20	1.58		3.50	1.07	
Days since last CC <sup>4</sup>	-0.03	0.12	0.7861	-0.27	0.14	0.0653
HLMC <sup>5</sup>	0.12	0.04	0.0022	0.10	0.05	0.0307
MMR L/d <sup>6</sup>	0.05	0.02	0.0285	-0.004	0.01	0.5477
MMR L/d squared	-0.0002	0.00	0.0493			

<sup>1</sup>Samples collected during 4-wk pre/post circuit cleaning study on VA farms only

<sup>2</sup>Aerobic plate count (log<sub>10</sub> cfu/ml)

<sup>3</sup>Coliform count (log<sub>10</sub> cfu/ml)

<sup>4</sup>Circuit cleaning

<sup>5</sup>Hours since last mixer/heat exchanger cleaning

<sup>6</sup>Milk or milk replacer delivered on day of sample collection

Table 16. Effect of between machine variables on log reduction of bacteria<sup>1</sup> (n = 49)

Variable <sup>4</sup>	LRAPC <sup>2</sup> (R <sup>2</sup> = 0.12)			LRCCP <sup>3</sup> (R <sup>2</sup> = 0.12)		
	Estimate	SE	P value	Estimate	SE	P value
Intercept	-0.20	0.43		-2.10	1.07	
Mixer/heat exchanger cleanings/d	0.30	0.16	0.0626	0.41	0.24	0.0868
Circuit cleanings/wk			0.4448	0.34	0.15	0.0242
Detergent level (ml/L water)			0.2547			0.2107
Chlorine bleach use (1 = yes, 0 = no)	0.43	0.25	0.0940			0.2894
Silicone feeder hose (1 = yes, 0 = no)			0.9402			0.1834
Vinyl feeder hose (1 = yes, 0 = no)			0.4167			0.3631
Plastic feeder hose (1 = yes, 0 = no)			0.2379			0.5430

<sup>1</sup>Samples collected during 4-wk pre/post circuit cleaning study on VA farms only

<sup>2</sup>Log reduction of aerobic plate count

<sup>3</sup>Log reduction of coliform count

<sup>4</sup>Variables with no estimates were removed for lack of significance prior to final model

Variable	LRAPC <sup>2</sup>			LRCCP <sup>3</sup>		
	Estimate	SE	P value	Estimate	SE	P value
Intercept	-3.72	1.45		-3.03	1.93	
Days since last CC	0.05	0.11	0.6657	-0.25	0.16	0.1285
HLMC <sup>4</sup>	-0.16	0.07	0.0335	0.15	0.04	0.0018
MMR L/d <sup>5</sup>	0.07	0.03	0.0023	0.05	0.03	0.0747
HLMC squared	0.01	0.00	0.0006			
MMR L/d squared	-0.0002	0.00	0.0038	-0.0002	0.00	0.0845

<sup>1</sup>Samples collected during 4-wk pre/post circuit cleaning study on VA farms only

<sup>2</sup>Log reduction of aerobic plate count

<sup>3</sup>Log reduction of coliform count

<sup>4</sup>Hours since last mixer/heat exchanger cleaning

<sup>5</sup>Milk or milk replacer delivered on day of sample collection

Variable	Aerobic plate count (log <sub>10</sub> cfu/ml)			Coliform count (log <sub>10</sub> cfu/ml)		
	Estimate	SE	P value	Estimate	SE	P value
Intercept	4.63	0.71		4.97	1.10	
FH <sup>2</sup>	-1.66	0.62	0.0088	-2.03	0.83	0.0164
MH <sup>3</sup>	1.76	0.46	0.0002	-0.03	0.20	0.8868
M <sup>4</sup>	-0.10	0.11	0.3542	-0.46	0.17	0.0076
H <sup>5</sup>	0.15	0.16	0.3388	-0.02	0.24	0.9453
FH squared	0.58	0.17	0.0012	0.49	0.19	0.0091
FH*MH	-0.62	0.17	0.0003			

<sup>1</sup>Items were scored on 0 – 3 scale, with 3 being the cleanest

<sup>2</sup>Feeder hose

<sup>3</sup>Mixer hose

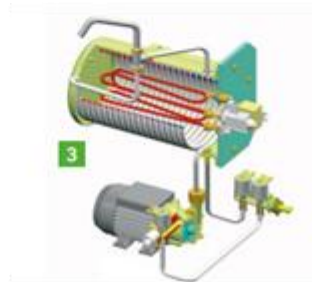
<sup>4</sup>Mixer

<sup>5</sup>Hopper



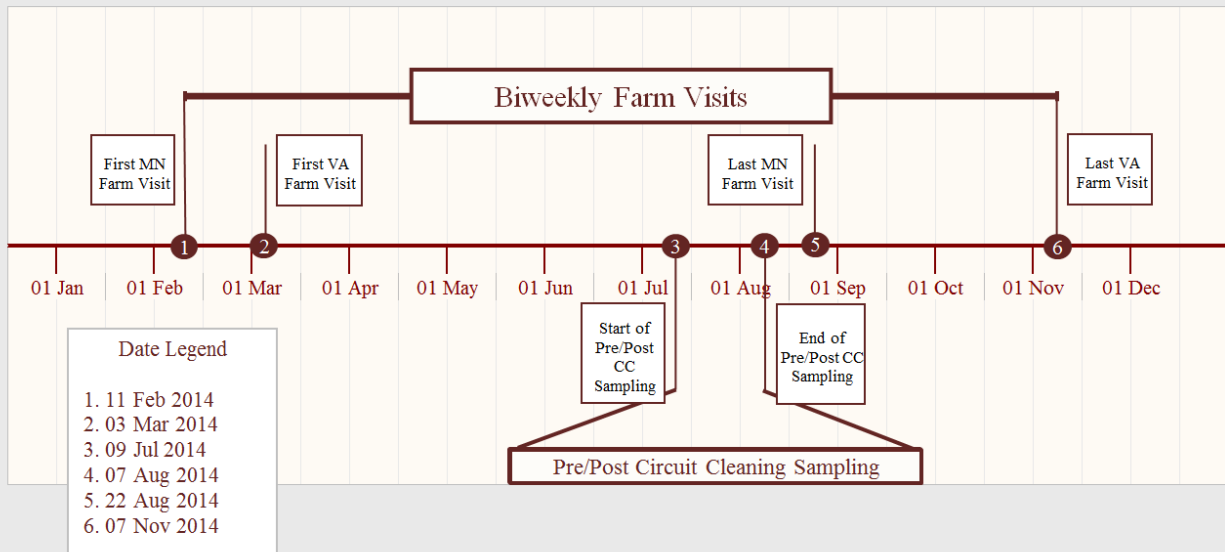
Figure 2. Automated calf feeder and optional heat exchanger

Adapted from <http://www.foerster-technik.de/>



- 1 Intensive mixer
- 2 Suction hose
- 3 Heat exchanger
- 4 Milk powder hopper

Figure 3. Sampling Timeline



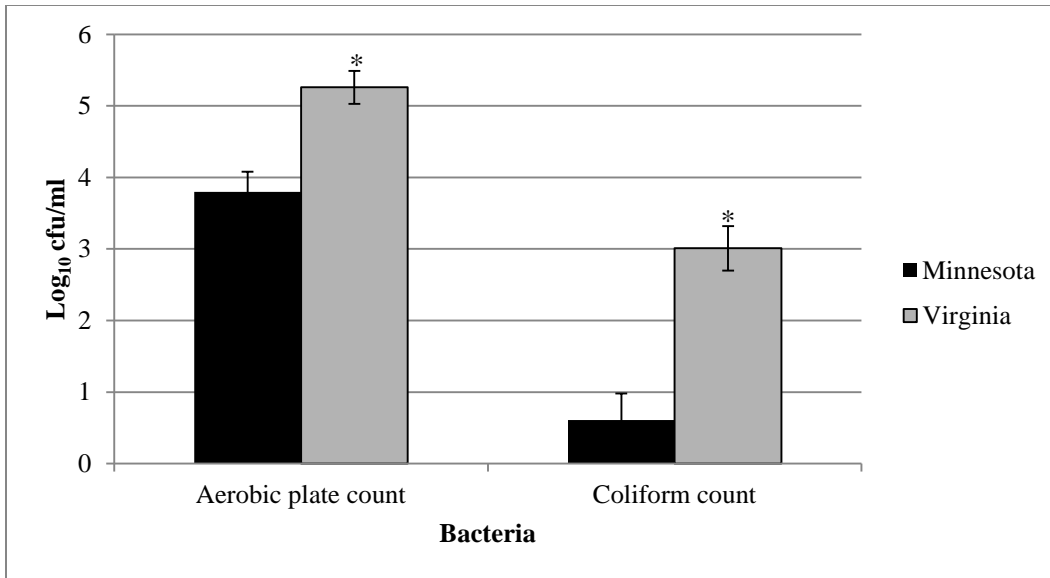


Figure 4. LS mean bacterial counts by state from biweekly farm visits

\* $P < 0.01$

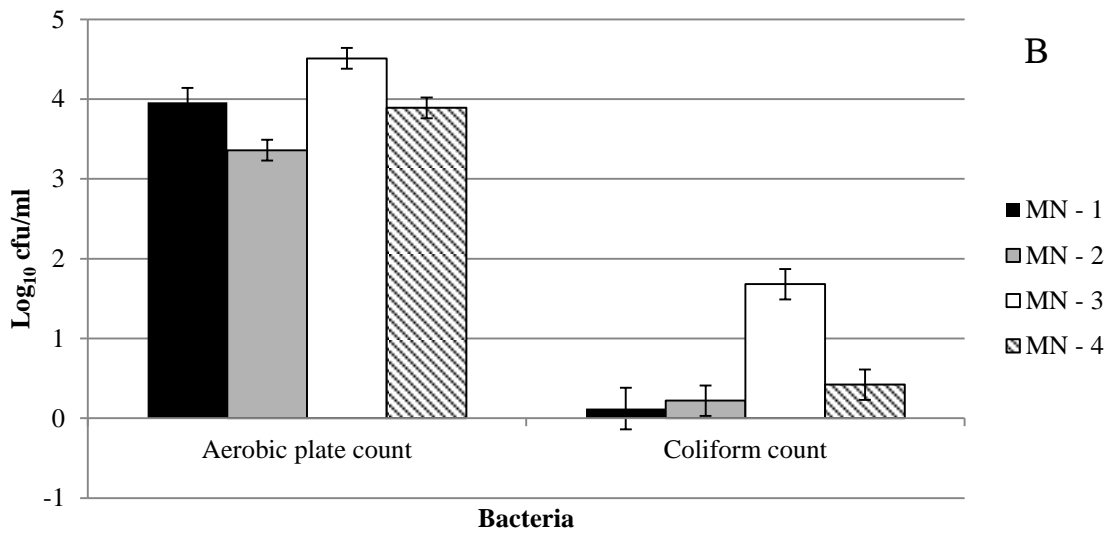
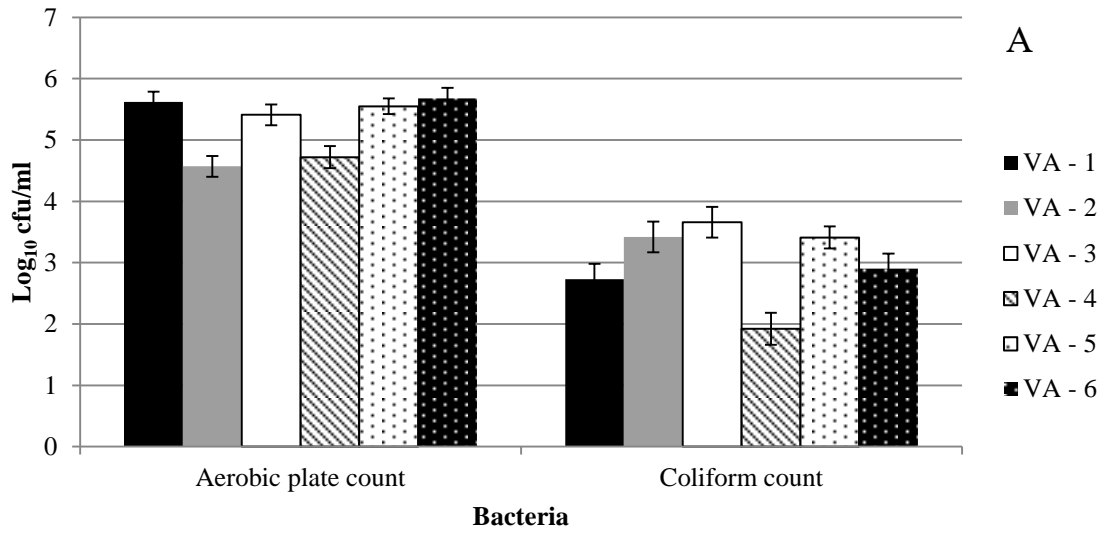


Figure 5. LS mean bacterial counts by farm (A) Virginia farms (B) Minnesota farms

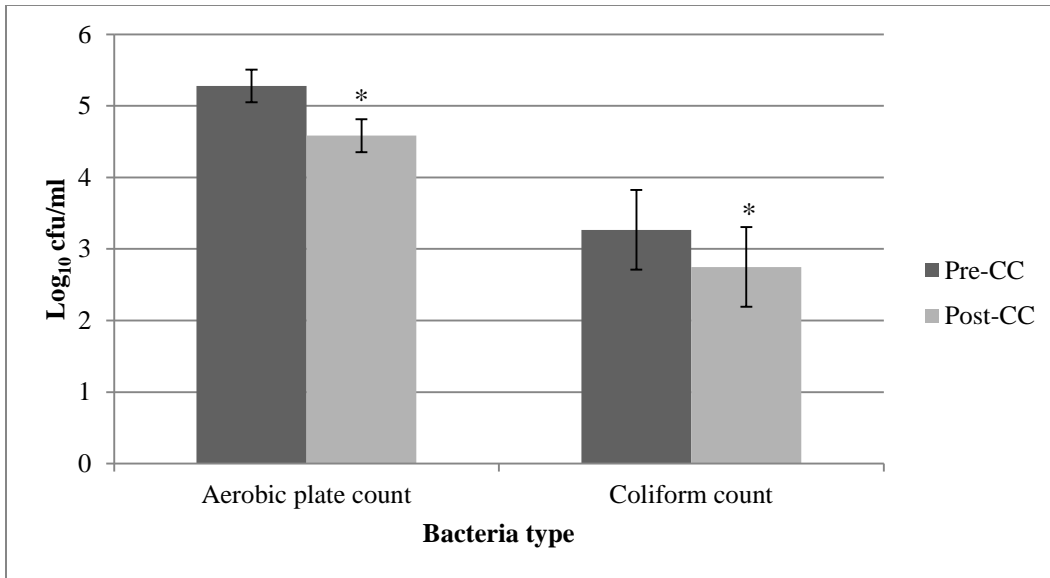


Figure 6. LS mean pre- and post-circuit cleaning (CC) bacterial counts  
 Samples collected during 4-wk pre/post circuit cleaning study on VA farms only  
 \* $P < 0.01$

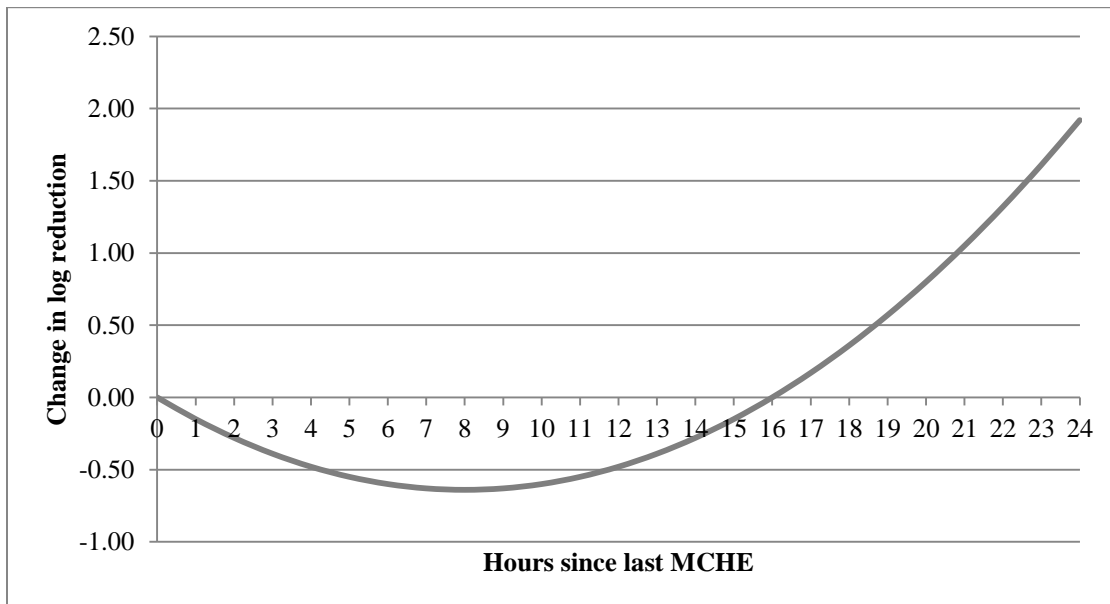


Figure 7. Change in log reduction of aerobic plate count for a 1 h delay at each hour since last mixer/heat exchanger cleaning (MCHE)

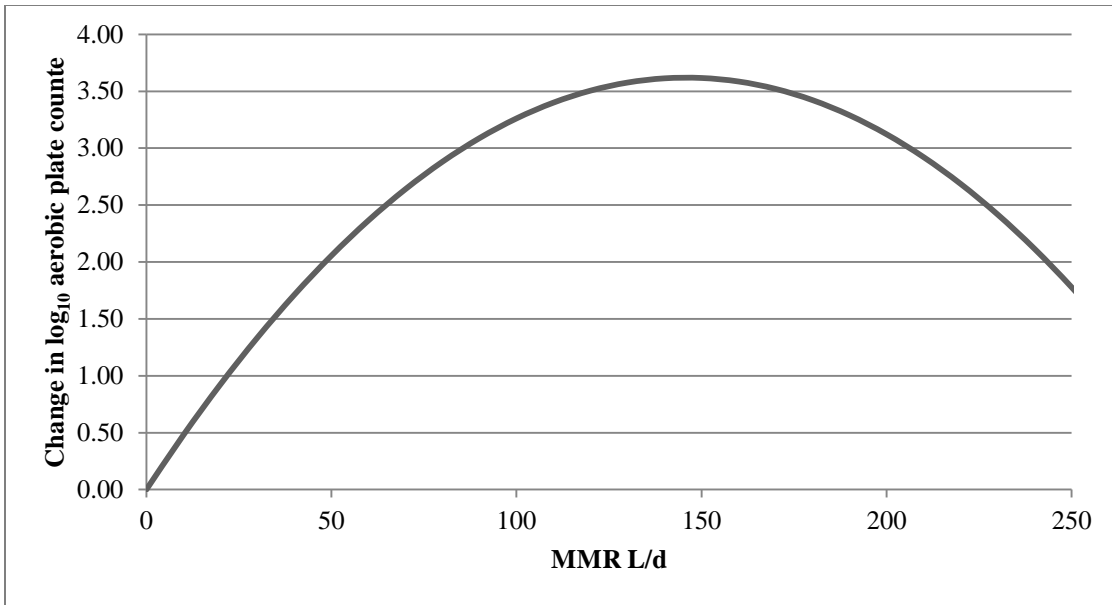


Figure 8. Change in aerobic plate count for 1 L change in milk or milk replacer delivered on day of sample collection (MMR L/d)

## **CHAPTER 3: VARIATION IN SOLIDS CONCENTRATIONS DELIVERED BY AUTOMATED FEEDER**

### **ABSTRACT**

The objective was to describe the variation in total solids concentrations of reconstituted milk replacer (MR) delivered by automated feeders. Milk replacer samples were collected biweekly from sophisticated automated feeders on 6 Virginia farms and weekly from 2 Minnesota farms for 26 to 28 wk. Total solids concentration was estimated for MR samples with optical and digital refractometers. Automated feeders delivered 12.26% of MR samples > 2% over and 25.71% > 2% under target solids concentration. Failure to routinely calibrate feeders likely caused variation, but was not investigated in the current study. Optical and digital refractometers were highly correlated with each other ( $R^2 = 0.96$ ), and can be used by producers to monitor total solids in MR delivered by automated feeder.



## **INTRODUCTION**

Consistent feeding of milk or milk replacer to dairy calves is essential to a successful nutrition program. Variation in solids concentrations may lead to gastrointestinal disease and reduced performance.

In waste milk feeding programs, variations in total solids is commonly observed. In contrast, solids concentration is more easily controlled in traditional milk replacer feeding. Automated calf feeders are designed to precisely measure powder and water to deliver consistent milk replacer concentrations at a specified temperature and avoid human error. However, machine calibration errors, changes in milk replacer composition, or machine obstructions may decrease accuracy. Research has not been conducted on variation in milk replacer delivered to calves via automated feeder. The objective of this study was to assess variation in total solids of such feed.

## REVIEW OF LITERATURE

### *TARGET DRY MATTER CONTENT OF MILK AND MILK REPLACER*

The national average solids nonfat and fat percentages for US milk production in 2010 were 8.81 and 3.66%, respectively, making the average solids content of milk 12.47% (Hoard's Dairyman, 2012). The solids content of nonsaleable milk, commonly known as waste milk, has considerable amounts of variation. Moore et al. (2009) collected waste milk samples from 12 dairies, estimating the percent solids with a Brix refractometer. Waste milk from farms in the study was intended to feed calves on a single calf ranch. Total solids (TS) ranged from 5.1 to 13.5% for the 12 samples collected, with an average of 11.1%. Variation in TS was also observed in a larger number of pasteurized waste milk samples from the same farm by Machado (2011), with a range of approximately 9 to 13% and a mean of 11.6%. Scott (2006) measured TS at 10 min intervals within the same feeding of pasteurized waste milk and found a range in LS means of approximately 1%. The variation in solids of waste milk reflects the inconsistent nature of the waste milk supply on a farm. Waste milk may be from a large proportion of cows that have recently calved, resulting in high solids, or may conversely be from a high proportion of mastitic cows, causing the pooled milk to be low in solids. Water used to flush milk lines also may decrease solids in waste milk (Machado, 2011).

There is potential for more control over solids concentrations when milk replacer is being fed instead of waste milk. Industry recommendations for the total solids of reconstituted MR are within a range of 10 to 15% (Davis and Drackley, 1998). According to Davis and Drackley (1998), most milk replacers used in the US are recommended to be fed at 9.2 to 12.3% solids. Förster Technik automated calf feeders have a default setting of 150 g powder plus 1,000 g water, for a solids content of 13.04%. For winter feeding, when nutrient requirements are higher

than normal, McGuirk (2014) suggested feeding milk replacer at approximately 15% solids, but never exceeding 18%. However, Davis and Drackley (1998) stated that during winter, feeding milk replacer at a formulation of up to 25% solids is acceptable and should not result in significant scouring.

### ***IMPORTANCE OF CONSISTENT MEALS FOR PREWEANED CALVES***

It is widely perceived that variation in milk or milk replacer delivery may limit performance of preweaned calves, but researchers have not investigated the effects of variable MR concentrations on calf health or growth. However, Hill et al. (2008) compared the effect of feeding inconsistent volumes of milk replacer to feeding fixed volumes daily. While each milk replacer treatment was mixed and fed at a consistent concentration of 148 g DM/L water, the calves on the variable treatment received a randomly selected level of 0.545, 0.754, 0.681, 0.817, or 0.608 g of milk replacer powder/d, with each level being fed on 1 d within each 5 d period. Calves on the fixed treatment received 0.681 g/d during this period. Following each 5 d test period, all calves received 0.681 g/d for 2 d to standardize gut fill that may have affected calf weights. Calves on the fixed treatment had greater ADG, starter intake, and feed efficiency than calves fed variable volumes of milk replacer ( $P < 0.05$ ), highlighting the importance of consistent feeding practices during preweaning (Hill et al., 2008).

McGuirk (2014) identified inconsistent delivery of feed as a major risk factor for abomasal disorders in dairy calves. Inconsistencies include variability in the feed solids concentrations, temperature, ingredients, additives, amount, and meal frequency (McGuirk, 2014). An acceptable goal for maintaining consistency between feedings is to remain within 1% solids between calves within a single feeding (McGuirk, 2014). Logically, minimal variation should occur between feedings as well.

The feeding of high DM concentrations in reconstituted milk replacer often gets blamed for calf scouring. However, this effect has not been consistently shown in research. Whereas Jenny et al. (1978) found a higher incidence and longer duration of scours in calves fed a milk replacer DM of 20% compared to calves fed 10% ( $P < 0.01$ ), contradictory results have been found in other studies. In a 3 x 3 factorial study, Stiles et al. (1974) fed milk replacer in 3 solids concentrations treatments of 6 to 8, 8 to 10, and 10 to 12% and 3 volume treatments of 5 to 7.5, 7.5 to 10, and 10 to 12.5% body weight (BW) over a 3 wk period. Solids concentration caused few differences in calf performance. However, calves on the high and intermediate solids treatment had less severe incidences of scours than calves fed the lowest solids concentration ( $P < 0.05$ ) (Stiles et al., 1974). Despite this, calves on the high volume treatment had more scours events ( $P < 0.05$ ) than calves on the low and medium treatments, from which the authors concluded that high levels of DM caused scours (Stiles et al., 1974). Drackley et al. (1996) compared the performance of Jersey calves fed milk replacer at concentrations of 12.5 or 15.5% solids and found no differences in fecal scores. Instead of the actual grams of solids fed, perhaps fluctuations in solids concentrations between feedings contribute to calf scouring due to gastrointestinal upset.

In addition to the implications for enteric disorders in calves, inconsistent delivery of milk replacer also may have economic disadvantages. Investing in a high plane of nutrition for calves can be beneficial to calf growth and performance. However, setbacks caused by variation in feed delivery could conceivably reverse such benefits. Lost opportunities for improved calf growth and the costs associated with treating scouring calves may hinder to the profitability of an inconsistent calf feeding program.

## ***AUTOMATED FEEDERS AND SOLIDS CONCENTRATIONS***

A potential advantage of automated calf feeders is that they are designed to accurately and precisely mix milk or milk replacer meals for a designated concentration of solids. Many sophisticated automated feeders feature a scale under the mixer, which weighs milk replacer powder and water prior to mixing. These machines may also auto-calibrate and can be manually calibrated by the producer to maintain precision and accuracy. It is worth noting that limited research has been done on the consistency of these machines in feed delivery.

Differences in the manufacturing process of milk replacer may influence mixing ability and lead to inconsistent concentrations. Agglomerated milk replacers, which are made by encasing fat droplets in protein and lactose, are known to have reduced clumping and are more easily mixed with water than non-agglomerated milk replacers (Davis and Drackley, 1998). Milk replacer powders of different brands or ingredients also may differ in their density or DM content and require re-calibration to maintain feeder accuracy.

Despite these factors contributing to variability, it is likely that calves fed with automated feeders receive more consistent solids concentrations compared to calves receiving milk replacer mixed by hand. Using a plastic cup provided in a standard bag of milk replacer, Penn State University students participated in a study in which they each prepared milk replacer for one calf according to label instructions, with the intention of delivering a formulation of 13% solids. While not a controlled study, the results demonstrated the variation that may occur on farm in the preparation of milk replacer. Forty-one samples were mixed, with a range in TS of 6 to 14.5%, a mean of 9.6%, and a standard deviation of 2.3% (Heinrichs, 2014).

## ***MONITORING SOLIDS CONCENTRATIONS***

Refractometers have multiple applications for monitoring aspects of the calf program. The Brix refractometer was originally used for measuring sucrose concentrations in beverages or liquid foods such as wine or syrup. This tool measures the refractive index and reports it in Brix, which is equal to 1 g sucrose per 100 g liquid (Moore et al., 2009). For use in liquids not containing sucrose, the % Brix reading can be used as an estimate of % TS (Deelen et al., 2014). On dairies, Brix refractometry is a convenient method of monitoring colostrum quality. Biemann et al. (2010) compared the use of optical and digital refractometers to radial immunodiffusion (RID) assays in measuring Ig concentrations in colostrum. Readings from both types of refractometers were closely related to the RID measurements, with correlations of 0.71 ( $P < 0.001$ ) and 0.73 ( $P < 0.001$ ) for optical refractometer and digital refractometer and RID values, respectively (Biemann et al., 2010). In the same study, no difference was seen in the refractometer readings between fresh and frozen samples measured with optical and digital refractometers ( $r = 0.98$ ,  $P < 0.001$ ;  $r = 0.97$ ,  $P < 0.001$ ) (Biemann et al., 2010).

Brix refractometers are also helpful in monitoring solids concentrations in waste milk. Moore et al. (2009) compared refractometry to mid-infrared spectrophotometry in evaluating TS of waste milk samples. The values reported by the two methods were positively correlated ( $R^2 = 0.8657$ ,  $P = 0.003$ ), and the relationship found between the two was:

$$(\% \text{ TS measured by spectrophotometry}) = 0.9984 * (\text{Brix refractometer reading}) + 2.077.$$

These results suggest that a Brix refractometer reading for waste milk plus approximately 2% may be used as an estimate of TS % (Moore et al., 2009).

Despite research validating the use of a Brix refractometer in monitoring colostrum, passive transfer, and total solids of waste milk, refractometer readings of milk replacer have not

been studied in comparison to TS measurements determined by sample dehydration. For the development of an equation to estimate TS of milk replacer samples from refractometer readings, McGuirk (2014) suggested first formulating mixed milk replacer solutions of 10, 12, 14, and 16% solids and recording refractometer readings. Plotting TS percentages against the refractometer readings and finding the line of best fit will result in a usable equation for determining TS for samples of that milk replacer in the future by refractometry. Because different milk replacers contain varying ingredients and content of fat, protein, and carbohydrates, a unique equation should be developed for each type of milk replacer.

## MATERIALS AND METHODS

During VA farm visits, duplicate milk replacer samples were collected aseptically into 15 ml tubes at the end of the feeder hose from each feeder in use. If a calf was drinking from the feeder at the time of sample collection, the flow was interrupted to collect the sample from that portion. If a milk replacer portion had to be mixed to create a sample, the liquid was expelled for at least 3 s prior to collection to flush out any residual water present in the feeder hose. Samples were transported on ice until arrival to the Virginia Tech Mastitis & Immunology Laboratory where they were frozen at -20 °C. During MN farm visits, samples were collected in the same manner using 50 ml tubes. Single samples from MN were shipped overnight to the Virginia Tech Mastitis & Immunology Laboratory on ice in 2 shipments, checked to ensure they had not thawed, and were immediately stored at -20 °C.

Duplicates of VA samples plated for the enumeration of bacteria and original MN samples were thawed at room temperature and vortexed for 10 s, or until solids appeared to no longer be separated. Up to 3 drops of each sample were pipetted onto an optical Brix refractometer (VEE GEE Scientific, Inc., Kirkland, WA) and a digital refractometer (MISCO Refractometer, Solon, OH). This step was repeated after inverting the sample several times. If the 2 digital refractometer readings differed from each other by > 1% Brix, a third reading was done, and the first was deleted. Both refractometers were calibrated with distilled water before each session, and the digital refractometer was calibrated after each sample.

To use Brix refractometer readings to estimate total solids (TS), refractometer data were plotted against total solids as measured by drying. Milk replacer type (distinguished by brand, CP:Fat ratio, and protein ingredient content) was identified for each VA sample by as identified during farm visits. Following analysis by refractometry, samples with the most variable readings



within the same milk replacer type were selected, poured into pre-weighed aluminum pans, weighed, and placed in a forced air oven at  $60 \pm 5$  °C for up to 20 h, or until dried weights were constant. Dried samples + pans were weighed, and % TS was determined by:

$$\% \text{ TS} = \left[ \frac{(\text{dry sample} + \text{pan}) - \text{pan}}{(\text{wet sample} + \text{pan}) - \text{pan}} \right] * 100$$

Percent TS data were plotted on the x-axis and average refractometer readings were plotted on the y-axis to determine a line of best fit for both refractometer types and each milk replacer type. Equations determined by lines of best fit were used to estimate % TS on all samples of each milk replacer type, regardless of whether or not they were dried.

To summarize data, % TS means and standard deviations were calculated for each machine feeding milk replacer. To assess the level of variation in % TS, the proportion of samples that differed by > 2% from the target % TS was calculated for each of the same machines. Target % TS was determined by feeding plans recorded in surveys. If farms changed milk replacer concentrations between feeding plans, the portion record in Institute software was used to identify target % TS for each sample according to date. Farm VA-1 implemented several different feeding plans using different milk replacer concentrations between and within feeding plans, so target % TS was estimated by calculating the mean milk replacer concentration mixed by the machine during the study period (derived from portion record in Institute software).

## RESULTS AND DISCUSSION

Mean estimated total solids (TS) for machines feeding MR were 12.43 and 12.38% as estimated by optical and digital Brix refractometry. Table 19 shows mean TS for each machine sampled, and the proportion of samples that were > 2% over or under the target concentration according to machine settings. The mean proportion of samples > 2% over their target was 12.26%, and the mean proportion > 2% under their target was 25.71%. Therefore nearly 40% of samples were not within 2% of the target concentration. Not only were MR samples inaccurate, they were inconsistently so, demonstrating a large amount of variability among samples from the same machine.

Sophisticated automated feeders are designed to precisely weigh MR powder and water to achieve the concentration designated by the producer. Sophisticated feeders may or may not feature auto-calibration, but all have the capability of alerting producers of calibration errors. Manual calibration is necessary when an alert is triggered and should be performed routinely (bimonthly for auto-calibrated machines or weekly for non-auto-calibrated machines). Unfortunately, not all farms on the current study recorded calibration events, so calibration was not analyzed as a factor contributing to TS variability. However, lack of regular feeder calibration is the most likely explanation for inconsistent MR concentration.

Auto-calibration occurs during the mixing of powder and water, so minor interruptions in powder or water flow to the mixer should be recognized and corrected by the machine. Large changes in TS in a short period of time may be due to obstructions in the automated feeder blocking the flow of powder or water. To prevent this, producers should routinely circuit clean machines and check for foreign materials in the hopper or mixer.

Changes in MR powder could affect the accuracy of calibration as well. Different batches or brands of MR may differ in DM content or density. In the current study, farms VA-1, VA-3, and VA-4 changed their MR during the study more than other farms (3 to 4 times), and had the most variation in % TS, determined by standard deviation.

Sampling errors may have also contributed to the inaccuracy in solids concentrations observed. Although researchers and producers collecting samples were instructed to flush any water out of the feeders prior to sample collection, it is possible that residual water reduced solids in MR samples.

McGuirk (2014) cites inconsistent MR delivery as a major risk factor for abomasal disorders in preweaned calves, suggesting a goal of < 1% change in TS between meals. This study showed the mean standard deviations of samples to be over 2.6% TS, with a large proportion of samples outside of 2% of their target TS.

Regardless of inconsistent delivery, MR delivered at a concentration different from the intended concentration may have detrimental effects on the performance and profitability of the calf operation. Calves will consume fewer nutrients than expected if they receive MR at concentrations lower than intended. A calf receiving 6 L/d of 22:20 CP:Fat MR at a concentration 2% lower than expected will receive 4.39 Mcal of ME less than intended over an 8-wk preweaning period (Quigley, 2007).

Due to the potential for negative effects on calf performance caused by TS inconsistency and inaccuracy, producers should routinely monitor concentrations of MR delivered by automated feeders. In this study, readings from optical and digital refractometers were highly correlated ( $R^2 = 0.96$ ; Figure 9). In addition, both refractometers were highly correlated with % TS values determined by drying (correlations for specific MR types listed in Table 22).

Therefore, either type of refractometer is useful in monitoring MR concentrations. An optical refractometer is approximately one third the cost of a digital refractometer (approximately \$100 vs. \$300), but is slightly more difficult to read. A producer using automated feeders should monitor TS at least weekly to determine if calibration is necessary.

### ***CONCLUSION ON VARIATION IN SOLIDS CONCENTRATIONS***

Milk replacer delivered by sophisticated automated feeders did not consistently deliver accurate concentrations of solids. Infrequent machine calibration and changes in milk replacer are likely the cause of variation. Because variability in solids may be detrimental to calf health and growth, producers should monitor milk replacer concentration routinely to determine when machine calibration is necessary. Producers should always calibrate feeders when switching to new batches of milk replacer as well. Optical and digital refractometers perform similarly in determining total solids in reconstituted milk replacer, therefore either could be used on-farm.

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Table 19. Total solids estimates of milk replacer by machine

Farm	Machine	Mean $\pm$ SD TS OPT <sup>1</sup>	Mean $\pm$ SD TS DIGI <sup>2</sup>	Target TS <sup>3</sup>	%Over <sup>4</sup>	%Under <sup>5</sup>	n
VA-1	1	13.03 $\pm$ 2.94	12.96 $\pm$ 3.05	13.69 <sup>6</sup>	N/A	N/A	40
VA-2	1	15.21 $\pm$ 1.70	15.10 $\pm$ 1.96	13.79	43.64	1.82	55
VA-3	1	11.92 $\pm$ 2.85	11.52 $\pm$ 3.00	12.51	14.55	27.27	60
VA-4	1	12.14 $\pm$ 2.92	11.86 $\pm$ 2.92	13.04	7.14	23.81	42
VA-5	1	11.38 $\pm$ 2.29	11.42 $\pm$ 2.21	13.04	0.00	30.00	30
VA-5	2	11.97 $\pm$ 1.88	12.11 $\pm$ 1.85	13.04	0.00	26.67	30
VA-6	1	11.03 $\pm$ 2.09	10.88 $\pm$ 2.25	13.04	0.00	50.68	74
MN-1	1	11.20 $\pm$ 2.66	11.48 $\pm$ 2.45	13.79	5.66	45.28	53
MN-2	1	13.52 $\pm$ 1.56	13.82 $\pm$ 1.90	12.59 <sup>7</sup>	29.27	0.00	41
MN-2	2	13.12 $\pm$ 1.62	13.11 $\pm$ 1.76	12.59 <sup>8</sup>	15.56	11.11	45

<sup>1</sup>% Total solids estimated by optical refractometer reading

<sup>2</sup>% Total solids estimated by digital refractometer reading

<sup>3</sup>% Total solids according to machine setting

<sup>4</sup>% of samples > 2 percentage points above target

<sup>5</sup>% of samples > 2 percentage points below target

<sup>6</sup>VA-1 had variable concentration settings, so mean TS of delivered milk replacer was calculated from portion report in Institute software

<sup>7,8</sup>Changed from 13.04% on 19 Mar 2014

Table 20. Equations for estimating total solids from refractometer reading<sup>1</sup>

Milk replacer	Optical			Digital		
	Coefficient	Intercept	R <sup>2</sup>	Coefficient	Intercept	R <sup>2</sup>
ANC AM w/ Clarifly	1.04	-0.13	1.00	0.99	0.32	1.00
Blueprint 22:20	1.23	-1.71	0.92	1.13	-0.69	0.92
LOL Cow's Match	0.91	1.95	0.96	0.76	3.52	0.99
LOL Cow's Match Warm Front	1.25	-0.68	0.99	1.33	-1.62	0.99
LOL Experimental	1.19	-0.54	0.95	1.24	-1.17	0.97
Purina 22:20	1.10	-0.43	1.00	1.10	-0.22	1.00
Purina 25:20	1.07	1.56	0.93	1.03	1.91	0.92
Renaissance + Plasma	1.33	-0.84	0.97	1.40	-1.89	0.99
Renaissance 22:20 Classic	1.24	-0.85	0.73	1.33	-1.97	0.74

<sup>1</sup>Determined by plotting total solids determined by oven drying on x-axis and Brix reading by refractometer on y-axis, and finding line of best fit

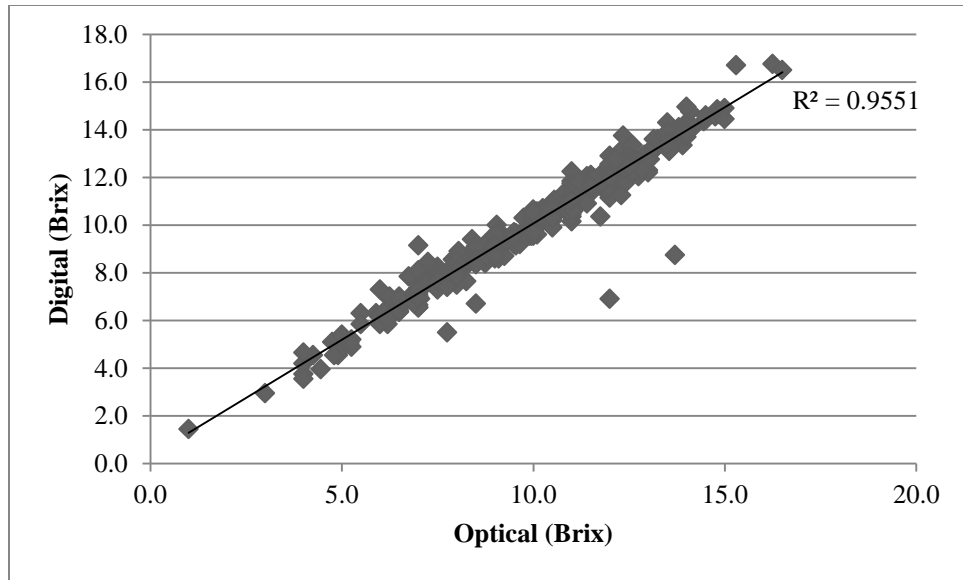


Figure 9. Relationship of Brix readings from optical and digital refractometers on same samples



## OVERALL CONCLUSIONS

Up to this point, automated feeder management recommendations, particularly those regarding sanitation, have been inconsistent and based on little research. A variety of management practices were represented on farms in this study, with differences observed on backgrounding, pen management, feeding, cleaning frequency, cleaning agents, and hose type.

Performing at least 4 mixer/heat exchanger cleanings per day is a simple way for producers to reduce bacteria concentrations in milk or milk replacer delivered by an automated calf feeder. Since it is an automatic cleaning cycle, it will be consistently implemented according to machine settings. Circuit cleaning is often considered the most critical step in sanitation management. However, this study demonstrated its inability to greatly reduce bacteria or maintain low bacterial counts.

Considerable variation in solids concentrations of milk replacer was observed in samples collected from automated feeders. Producers may rely too heavily on the machine to correct calibration errors, and therefore should routinely monitor total solids to identify variation and avoid calf illness or nutrient deficiencies that could be related to inaccurate total solids intake.

## APPENDICES

### A. Initial farm survey

FARM INFORMATION		
Farm name:	Manager name:	
Address:		
Phone:	Email:	
Herd size:	Breed:	
DHI herd code/ RAC code:		
Autofeeder manufacturer and model:		
CALF FACILITIES		
Style of barn:	<input type="checkbox"/> Three-sided	
	<input type="checkbox"/> Four-sided	
	<input type="checkbox"/> Hoop barn	
	<input type="checkbox"/> Other:	
Number of stalls:	Number of stalls per pen:	Stall width:
Number of calves per pen:	Number of autofeeder:	Number of calves per autofeeder:

Pen Number	Pen Dimension	Age of Calves in This Pen


Approximate space allotted to each calf (ft<sup>2</sup>):


Description of ventilation system:



Number of ducts:


Size of ducts:

Hole spacing:

Bedding score: 	0) Freshly bedded, sufficient dryness and depth
	1) Mostly dry, some isolated wet spots, acceptable depth
	2) Bedding is moist and brown in most areas
	3) Very wet/soiled bedding

Barn temperature:

Description of area in which autfeeder is housed:



**CALF MANAGEMENT**

**Housing before transfer to group pen**




<input type="checkbox"/> Outside	<input type="checkbox"/> Indoors
<input type="checkbox"/> Plastic hutch	<input type="checkbox"/> Wooden hutch

Other:


Space allotted to each calf:	Bedding:
------------------------------	----------

**Feeding**


Describe design and location of feeding trough for starter:



Condition of starter and trough:	0) Plenty of clean, fresh starter
	1) Plenty of starter, but not fresh
	2) Starter is very wet, old, and/or dirty
	3) Completely empty

Type(s) of waterers:	Calves per waterer:
Waterer cleanliness (check all that apply) 	0) Clean, clear water
	1) Moderately cloudy
	2) Very cloudy water; waterer itself is dirty
	3) Feces or mud in water; no access to clean water

<b>AUTOFEEDER MANAGEMENT</b>
<b>Sanitation</b>
Water temperature in sanitation cycle:
Sanitation settings on autofeeder:

Rate the cleanliness of the following areas (0 = Very dirty, 3 = Very clean)				
				
Feeding area	0	1	2	3
Hose connecting mixer to nipple	0	1	2	3
Hose inside autofeeder	0	1	2	3
Mixer	0	1	2	3
Hopper	0	1	2	3
Outside of autofeeder	0	1	2	3
Area surrounding autofeeder	0	1	2	3
Milk replacer handling	0	1	2	3
Pasteurizer	0	1	2	3
Waste or whole milk tank	0	1	2	3
Temperature in location of autofeeder:				

<b>Alarm Settings</b>
Feed consumption:
Drinking speed:
Breaks:
Robbery:
Additive issues:


<b>Feeder Status (Status CF)</b>
----------------------------------

Feeder 1:	Time of last data transfer:
	Status:
Feeder 2:	Time of last data transfer:
	Status:


<b>Employee Management</b>	
How do employees monitor calves using autofeeder?	
How do employees communicate to others on different shifts?	Tools used (e.g. white board):
Are protocols posted and used?	Describe:

B. Routine observations

<b>FARM INFORMATION</b>	
Farm name:	Manager name:
Address:	
Phone:	Email:
Herd size:	Breed:
DHI herd code/ RAC code:	
<b>CALF MANAGEMENT</b>	
Number of calves per pen:	Number of calves per autofeeder:
Air movement:	
Barn temperature:	

Rate the condition of the following (0 = Very dirty, 3 = Very clean)				
				
Bedding	0	1	2	3
Calf starter	0	1	2	3
Waterers	0	1	2	3

<b>AUTOFEEDER MANAGEMENT</b>	
<b>Sanitation</b>	
Water temperature in sanitation cycle:	
Date & time of last cleaning:	
Sanitation settings on autofeeder:	

Rate the cleanliness of the following areas (0 = Very dirty, 3 = Very clean)				
				
Feeding area	0	1	2	3
Hose connecting mixer to nipple	0	1	2	3
Hose inside autofeeder	0	1	2	3
Mixer	0	1	2	3
Hopper	0	1	2	3
Outside of autofeeder	0	1	2	3
Area surrounding autofeeder	0	1	2	3
Milk replacer handling	0	1	2	3

Pasteurizer	0	1	2	3
Waste or whole milk tank	0	1	2	3
Temperature in location of autofeeder:				

<b>Feeder Status (Status CF)</b>	
Feeder 1:	Time of last data transfer:
	Status:
Feeder 2:	Time of last data transfer:
	Status:

<b>Employee Management</b>	
How do employees monitor calves using autofeeder?	
How do employees communicate to others on different shifts?	Tools used (e.g. white board):
Are protocols posted and used?	Describe:
Any changes in calf employees?	

C. Feeding plans in place during study

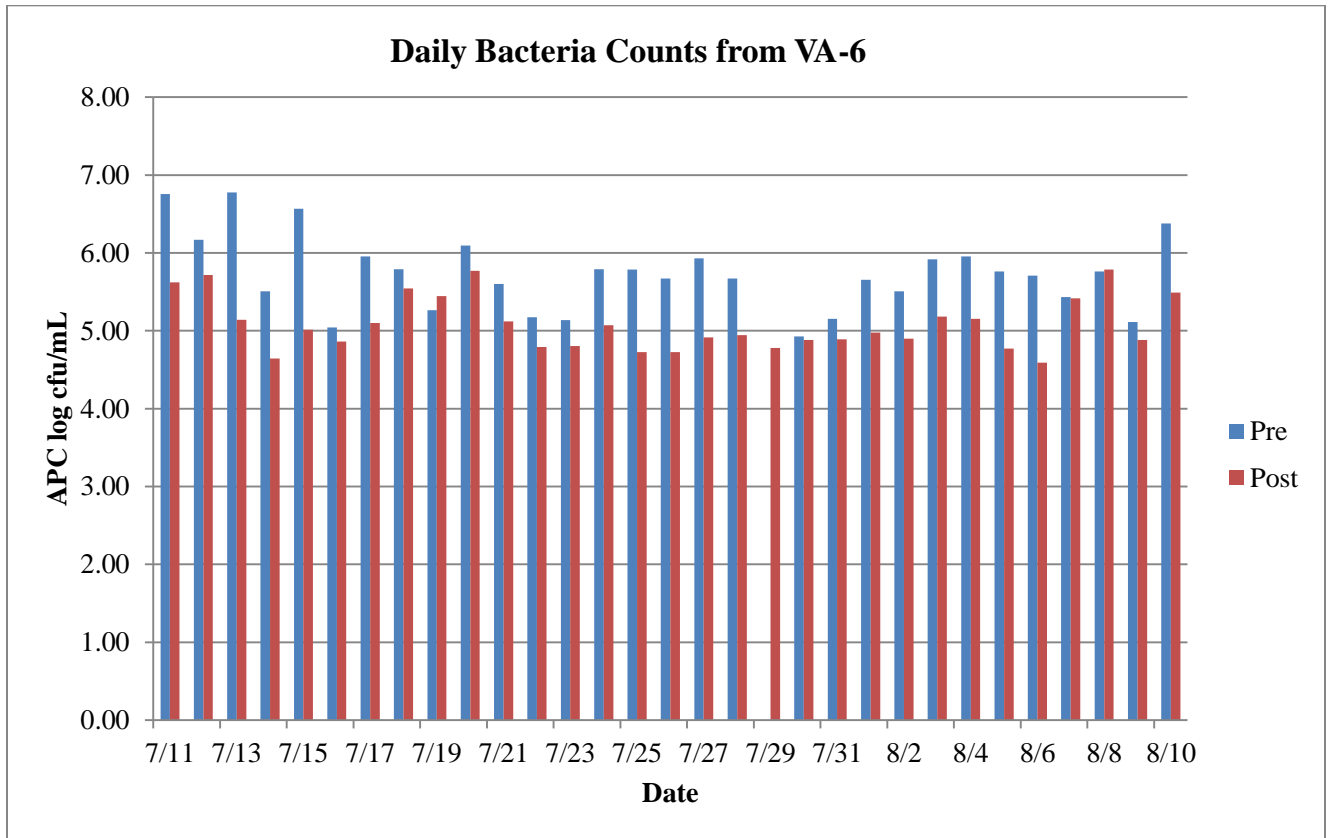
Farm	Plan (date retrieved) <sup>1</sup>	Allotment (L/d)			Concentration (g)			Meal size (L)		
		Days	Start	Final	Days	Start	Final	Days	Min	Max
VA-1	A (1/29/14)	18	4.0	16.0	39	150	160	7	1.5	2.5
VA-1	A (1/29/14)	28	16.0	16.0	7	160	160	8	2.0	3.0
VA-1	A (1/29/14)	10	16.0	0.0	10	160	160	9	2.5	3.5
VA-1	A (1/29/14)							25	3.0	4.0
VA-1	A (1/29/14)							7	1.5	2.0
VA-1	B (1/29/14)	7	5.7	9.0	7	140	164	7	1.8	2.7
VA-1	B (1/29/14)	41	9.0	9.0	41	164	164	35	1.8	3.0
VA-1	B (1/29/14)	2	9.0	4.4	2	164	155	7	2.2	3.0
VA-1	B (1/29/14)	6	4.4	4.4	6	155	155	7	2.2	2.7
VA-1	B (1/29/14)									
VA-1	C (1/29/14)	7	4.7	7.0	56	162	162	7	1.5	2.2
VA-1	C (1/29/14)	41	7.0	7.0				35	1.6	2.2
VA-1	C (1/29/14)	2	7.0	3.5				7	2.0	2.2
VA-1	C (1/29/14)	6	3.5	3.5				7	1.6	1.8
VA-1	C (1/29/14)									
VA-1	D (1/29/14)	14	4.0	11.0	21	150	165	7	1.5	2.0
VA-1	D (1/29/14)	35	11.0	11.0	35	165	165	8	2.0	2.5
VA-1	D (1/29/14)	7	11.0	0.0				9	2.5	3.0
VA-1	D (1/29/14)							25	3.0	3.5
VA-1	D (1/29/14)							7	1.5	2.0
VA-2	B	7	6.0	8.0	63	160	160	5	1.3	2.0
VA-2	B	34	8.0	8.0				37	1.5	2.5
VA-2	B	22	8.0	2.5				21	2.2	3.0
VA-3	A	3	6.0	9.0	50	143	143	43	1.5	2.5
VA-3	A	36	9.0	9.0				7	1.5	2.0
VA-3	A	2	9.0	4.5						
VA-4	A	5	5.0	6.0	56	150	150	5	2.0	2.0
VA-4	A	10	6.0	8.0				10	2.0	2.0
VA-4	A	30	8.0	8.0				30	1.5	2.5
VA-4	A	9	8.0	2.0				9	1.5	2.5
VA-5	A	6	5.0	6.0	56	150	150	5	1.0	2.0
VA-5	A	30	6.0	7.0				51	1.5	2.5
VA-5	A	10	7.0	4.5						
VA-5	A	10	4.5	2.0						



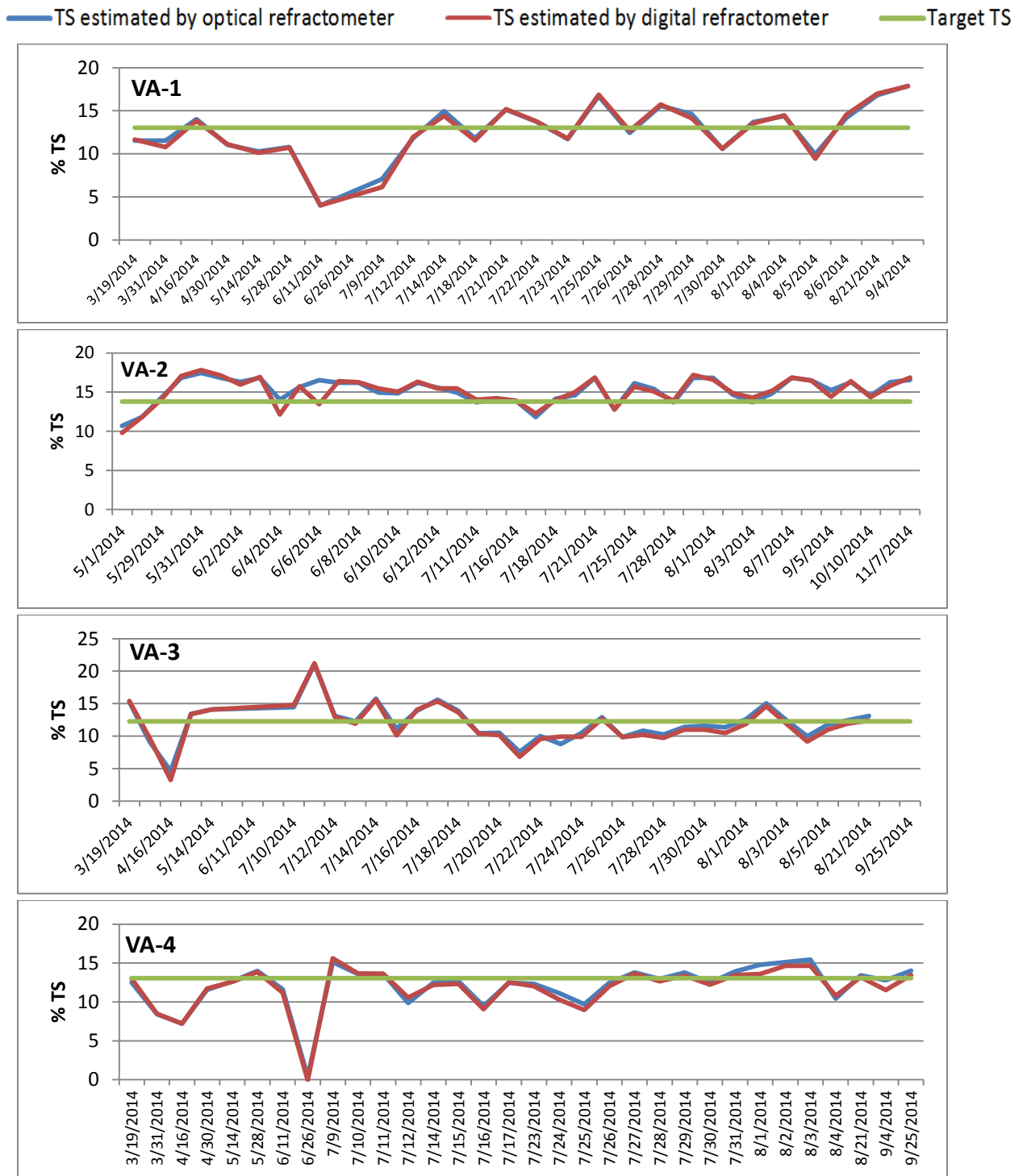
VA-6	A (6/2/14)	3	6.0	6.0	48	150	150	3	1.5	2.0
VA-6	A (6/2/14)	10	6.0	8.0				10	1.5	2.0
VA-6	A (6/2/14)	25	8.0	10.0				25	1.5	2.5
VA-6	A (6/2/14)	10	10.0	2.5				10	1.5	2.0
VA-6	B (6/2/14)	3	4.0	6.0	48	150	150	3	1.0	2.0
VA-6	B (6/2/14)	10	6.0	8.0				10	1.0	2.0
VA-6	B (6/2/14)	25	8.0	10.0				25	1.0	2.0
VA-6	B (6/2/14)	10	10.0	2.5				10	1.0	2.0
VA-6	C (6/2/14)	3	6.0	7.0	48	150	150	3	1.5	2.0
VA-6	C (6/2/14)	7	7.0	10.0				7	1.5	2.5
VA-6	C (6/2/14)	28	10.0	12.0				28	1.5	2.5
VA-6	C (6/2/14)	10	12.0	2.5				10	1.5	2.0
VA-6	B (8/22/14)	3	6.0	7.0	48	150	150	3	1.0	2.0
VA-6	B (8/22/14)	7	7.0	10.0				7	1.0	2.0
VA-6	B (8/22/14)	28	10.0	12.0				28	1.0	2.0
VA-6	B (8/22/14)	10	12.0	2.5				10	1.0	2.0
MN-1	A	7	3.0	6.0	43	160	160	7	0.5	2
MN-1	A	10	6.0	6.0				10	0.5	2
MN-1	A	23	6.0	8.0				23	0.5	2
MN-1	A	3	8.0	5.0				3	0.5	2
MN-2	A	5	7.0	8.0	40	150	150	5	1.6	2.0
MN-2	A	28	8.0	8.0				28	1.6	5.0
MN-2	A	7	8.0	4.0				7	2.0	2.7
MN-2	A							0	2.0	2.5
MN-3	A	2	5.0	6.0	42	25 <sup>2</sup>	25	2	1.5	2.0
MN-3	A	5	6.0	10.0				5	1.5	2.0
MN-3	A	22	10.0	10.0				22	2.0	2.5
MN-3	A	8	10.0	5.0				8	2.5	3.0
MN-3	A	5	5.0	0.0				5	2.5	3.0
MN-4	A	3	6.0	7.0	N/A	N/A	N/A	7	0.2	2.0
MN-4	A	4	7.0	9.0				7	2.0	2.5
MN-4	A	31	9.0	9.0				41	2.5	3.0
MN-4	A	17	9.0	0.2						

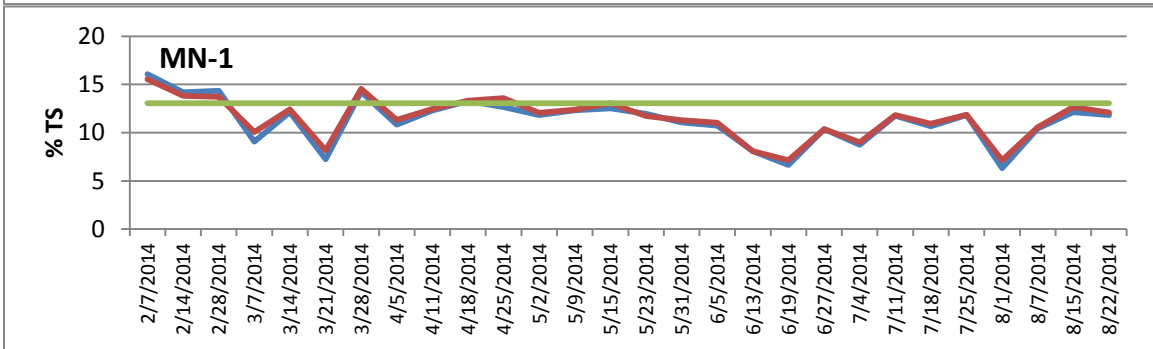
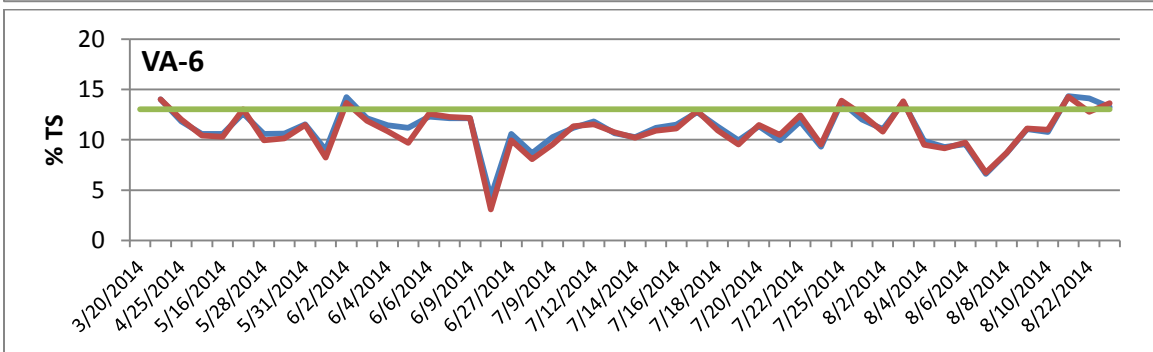
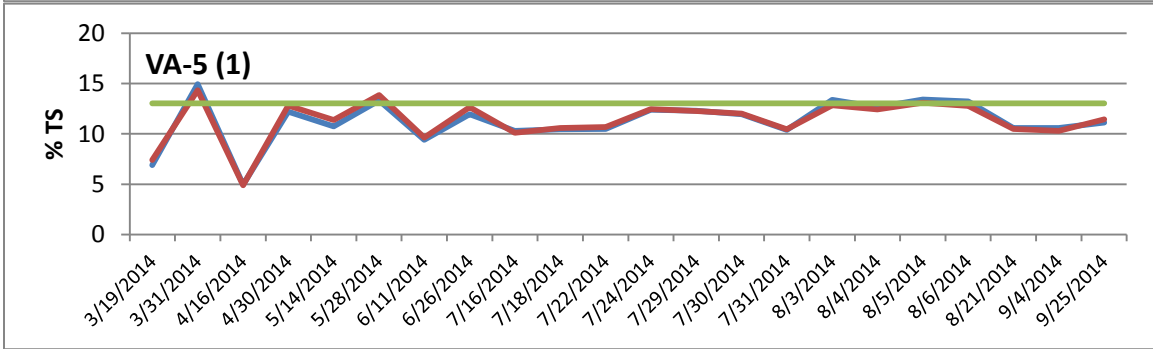
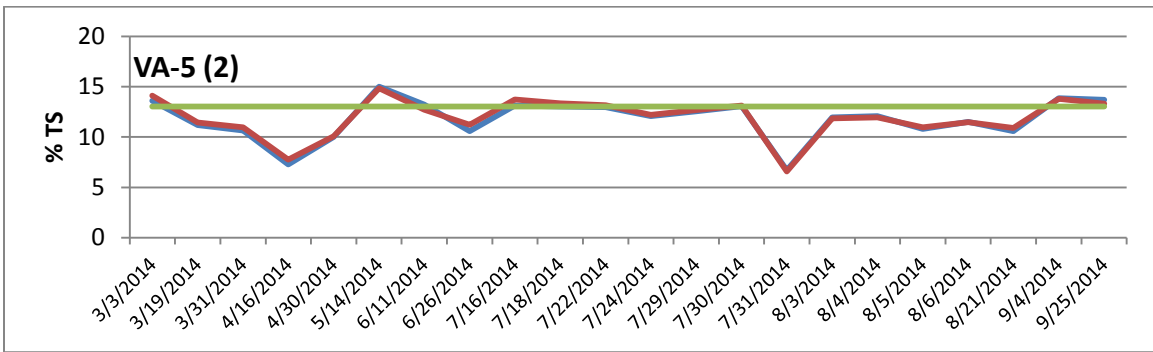
<sup>1</sup>Plans with no date remained constant throughout study, <sup>2</sup>Pasteurized waste milk balanced with 25 g milk replacer

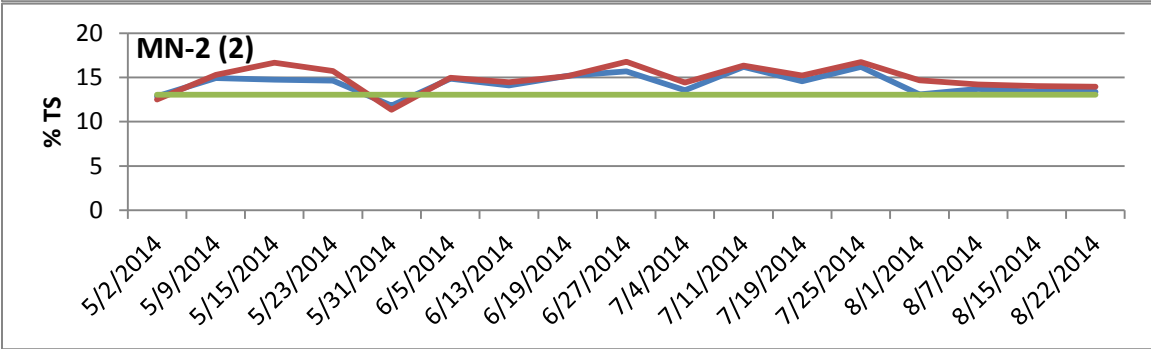
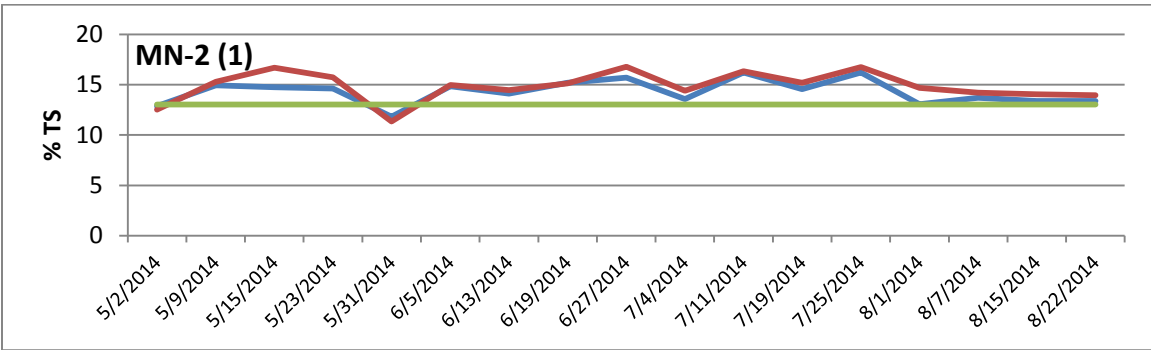
D. Daily aerobic plate counts from VA-6 during pre-/post-circuit cleaning sampling period



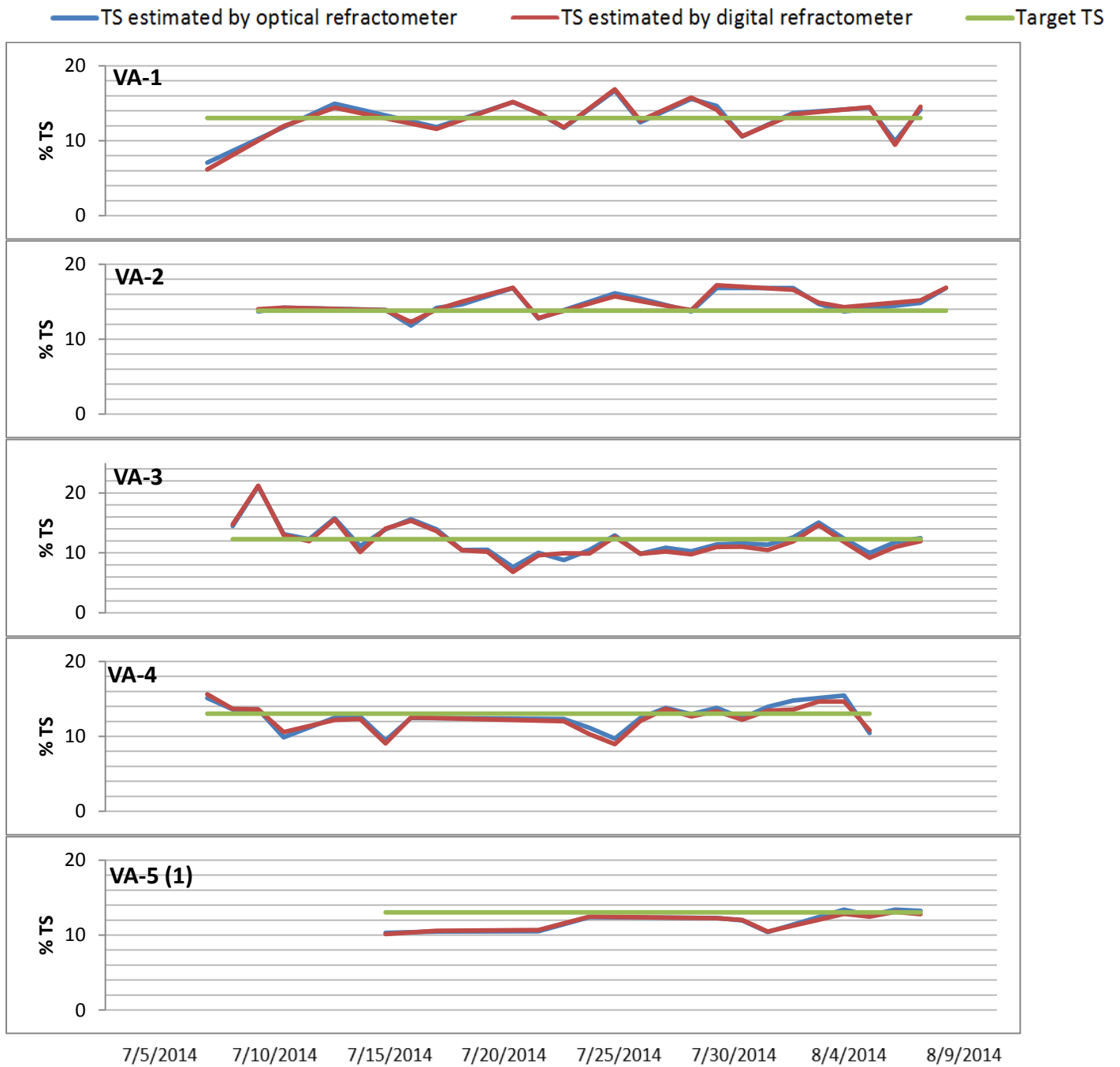
E. Sample-by-sample variation in total solids concentration by farm-machine

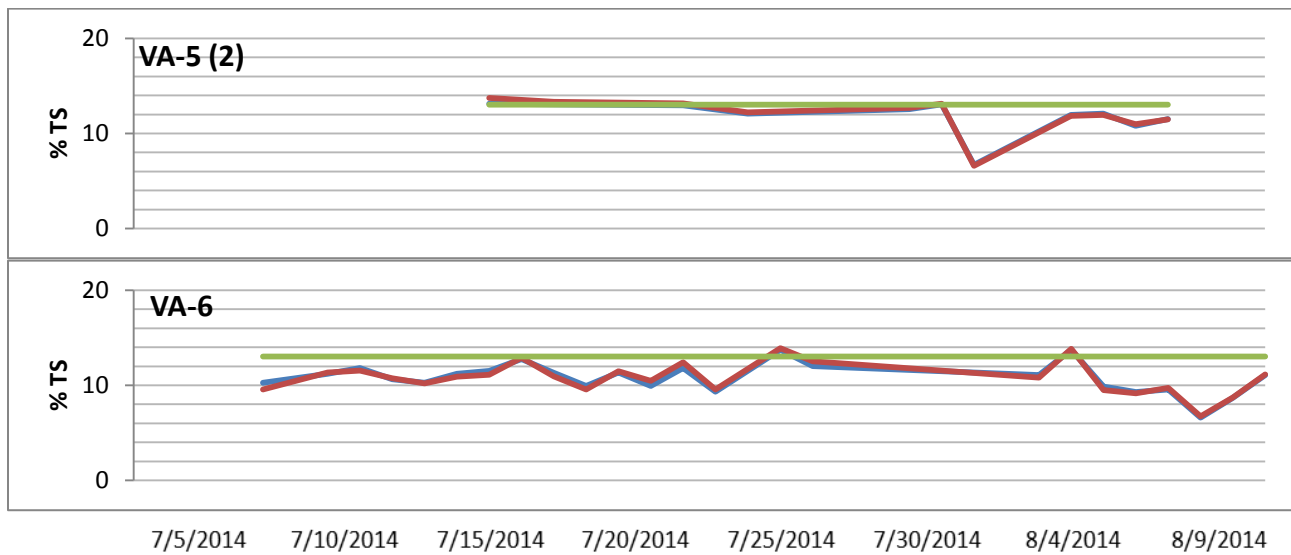






F. Daily variation in total solids during pre-/post-circuit cleaning sampling period





### G. Study observation periods by farm

Farm	Farm visits			Institute records <sup>1</sup>		
	Start date	end date	Days	Start date	End date	Days
VA-1	03.03.2014	25.09.2014	206	31.03.2014	25.09.2014	178
VA-2	01.05.2014	07.11.2014	190	01.05.2014	07.11.2014	190
VA-3	03.03.2014	25.09.2014	206	N/A	N/A	N/A
VA-4	19.03.2014	25.09.2014	190	16.04.2014	25.09.2014	162
VA-5	03.03.2014	25.09.2014	206	31.03.2014	25.09.2014	178
VA-6	03.03.2014	26.09.2014	206	26.03.2014	26.09.2014	183
MN-1	28.02.2014	22.08.2014	175	N/A	N/A	N/A
MN-2	14.02.2014	22.08.2014	189	07.03.2014	22.08.2014	168
MN-3	11.02.2014	19.08.2014	189	25.06.2014	19.08.2014	55
MN-4	04.03.2014	19.08.2014	168	04.03.2014	19.08.2014	168

<sup>1</sup>Time period during which the Institute software system recorded autofeeder data