Effect of Transition to the Dry Period on Fecal Shedding of Coliform Bacteria in Dairy Cows

Douglas Liebe

Department of Animal Sciences

Honors Research Thesis

The Ohio State University

Abstract

On well-managed dairy farms, environmental mastitis leading to clinical mastitis (CM) in dairy cows is common, and the most likely cause identified is certain gram-negative bacteria. Clinical mastitis is a very costly disease for farmers, limiting milk production and saleable milk, as well as negatively affecting milk quality. Previous studies have shown that the dry period is a critical time in the development of new intramammary infections that persist into the next lactation. In some beef feedlot studies, large increases in grain have resulted in increased fecal shedding of E. coli bacteria. Cows are fed primarily a high grain diet and a forage-based diet in lactation and the dry period, respectively. Lactating cows are fed high levels of grain to support high milk yields. The high level of grain in late lactation may be associated with higher fecal shedding of bacteria compared to the dry period when lower levels of grain are fed. The objective of this study was to quantify the effect of a rapid change in grain levels in the diet by measuring fecal shedding in dairy cows before and after dry-off. Fecal samples, from cows at two dairy farms either one week before drying off (n = 25) or two weeks into the dry period (n = 30), were taken rectally and immediately plated on MacConkey agar plates, selecting for coliform bacteria. The numbers of coliform

bacteria in the feces of cows were measured to quantify the shift in bacterial shedding counts as affected by the transition between lactation and dry-off. Results showed no change in coliform levels between late-lactation and the dry period ($P = 0.78$). Because high variability of coliform fecal shedding was seen between cows on each farm, detection of changes associated with grain feeding were likely masked; however, additional studies following cows through the transition from lactation to dry-off are needed to confirm.

Introduction

Clinical mastitis (CM) caused by environmental pathogens is the most common type of mastitis on well-managed dairy farms[1]. Environmental mastitis can be caused by any pathogen whose primary reservoir is the environment in which the cows live. This type of mastitis is categorized separately from contagious mastitis because contagious pathogens primarily live in the udder and are transmitted from cow to cow[2]. Intramammary infection (IMI) is directly correlated to the presence of mastitis pathogens on the teat end, and increased rates of CM occur with increased teat-end exposure to pathogens in bedding[3]. Coliform bacteria are a primary cause of environmental cases of CM, especially as confinement housing has increased in popularity. The proportion of CM cases related to coliform bacteria has been cited as 43.5, 40.0, and even 82.3% of cases in low somatic cell count (SCC) herds[4,5]. Research on feedlot cattle fed a high grain diet revealed that fecal shedding of bacteria increases with increased grain intake[6]. In the colon and cecum of ruminants, bacteria thrive on starch, fermenting it and releasing volatile fatty acids (VFA). On dairy farms, lactating cows are fed high grain diets to meet the energy demands for producing milk, while dry cows are fed primarily a forage-based diet.

Previous studies identified the dry period as having a significant effect on CM in the subsequent lactation. Pinedo et al. (2012) found an odds ratio (OR) of 2.7 for the chances of developing CM in the first 60 days of the subsequent lactation when environmental pathogens were isolated in the milk at dry-off, as well as an OR of 10.3 for cows with gram-negative bacteria specifically[7]. By better quantifying the effect of shifts from a high-grain to a forage-based diet, we may be better able to assess feeding strategies during times of increased risk of IMI, as well as possibly lower CM in the subsequent lactation.

Problem Identification and Justification

Mastitis is the most common disease in dairy cattle. Forty percent of CM cases are a result of gram-negative bacteria[8]. Of these bacteria, coliform bacteria, such as *E. coli* and *Klebsiella* spp., are observed most frequently[2,8]. Coliform bacteria are present in organic bedding material, especially wood by-products[3]. Studies have indicated that feces are the most likely source of these coliform bacteria, due to the interfacing of fecal matter and bedding, as well as the high rates of bacterial shedding on well-maintained dairy farms[8].

Environmental mastitis is very costly to dairy farmers. Without mastitis control programs, CM can lead to lower milk yield, poor udder health, increased treatment costs, dumped milk to avoid antibiotic residues, decreased milk quality, varied milk composition, and thus reduced profitability[9]. Pre-milking and post-milking teat disinfection and dry cow therapy reduce the risk of CM caused by contagious pathogens.[2] In fact, teat dipping can save the farmer approximately $75 per cow per lactation[9]. However, these methods prove to be ineffective at preventing environmental mastitis[2]. Clearly, researchers must identify ways to control pathogens in the cow's environment. The obvious problem is that a cow's environment is inherently unclean due to the constant fecal shedding of bacteria and the growth of bacteria in organic materials.

Previous studies suggest that the fecal shedding of bacteria is related to the diet of beef cattle[6,10]. Grain feeding has been found to increase acid-resistance in *E. coli,* a bacterium that is established in the colon and cecum of ruminants[11]. Studies indicate that reducing starch load may reduce fecal shedding, and this practice could be used as a means of reducing *E. coli* in slaughterhouses[12]. Feedlot cattle fed mostly grain had approximately 1,000 times more *E. coli* per gram of feces than cattle fed hay[10]. The more bacteria thriving in the large intestine, the more that

are shed in the feces[10]. This clearly shows a relationship between grain feeding and bacteria entering the environment through fecal shedding.

With the increased interest in the effect of varying diets on fecal shedding, the need for data on the effect of changes in a lactating dairy cow's diet is obvious. So far, most data regarding fecal shedding of coliform bacteria has focused on feedlot cattle and reducing the presence of such bacteria in the slaughterhouse[6]. Confinement housing lends itself to an environment for the dairy cows where the udders are frequently subjected to the environment and the pathogens associated with it. Control of environmental pathogens is difficult[2], which leads to an increased rate of CM on dairy farms[1]. We proposed that the drastic lowering of grain in the diet at dry-off may significantly decrease shedding of bacteria.

Hypothesis and Objectives

The objective was to investigate the potential of adjusting grain consumption near dry-off to reduce fecal shedding in dairy cows, and ultimately to reduce environmental pathogens in feces. *It was hypothesized that as cows dry off and subsequently consume much less grain, the rate of fecal shedding of coliform bacteria will also decrease.* This hypothesis was tested through the execution of two distinct objectives. **Objective 1: Measure rate of fecal shedding of coliform bacteria in cows in late lactation and early dry period.** The working hypothesis for Objective 1 was that the number of colonies grown from fecal dilutions on plates would be significantly decreased in the dry cow samples compared to late lactation cows. **Objective 2: Measure milk SCC for animals on the trial to determine potential correlation with fecal shedding rates.** The working hypothesis for Objective 2 was that cows with a higher fecal shedding of coliforms would have higher milk SCC, thus a positive correlation.

Materials and Methods

Design: Two commercial dairy herds with Holstein cows in southcentral Ohio were used for this project (Farm A = 1300 cows and Farm B = 2250 cows). We selected 15 cows from each of the two farms that were approximately one week from drying off, as well as 15 cows from each farm that were approximately 2 weeks into the dry period. Lactating cows were fed a daily TMR ration, while dry cows were fed one forage-based dry-cow diet (Table 1). All cows had parity >1. We took fecal samples from all cows in the trial on each farm.

Animals in Study: 52 total cows were selected for this trial. An animal use protocol was approved by The Ohio State Institutional Animal Use and Care Committee (IACUC ID: 2016A00000078). All cows were selected randomly from a pool of cows fitting the aforementioned criteria. This ensured that cows had adjusted to their new diet before the second sampling date. All cows were milked 3x/day and are described in Table 2.

Fecal Samples: All fecal samples were taken directly from the rectum using individual palpation sleeves. Fecal samples were placed in containers on ice for transport back to the lab. Samples were immediately (within 1 hour) diluted in PBS solution (Pelan-Mattocks et al., 2000). Analyses of data for statistical differences were determined using PROC MIXED model of SAS (SAS Institute, Cary, NC). Differences were considered significant at $P < 0.05$ with trends noted at $0.05 < P < 0.10$.

Detailed Procedures: For Objective 1, collected fecal samples were immediately diluted in PBS solution (15 mM KH_2PO_4, 8 mM Na_2HPO_4, 137 mM NaCl, 2.6 mM KCl, pH 7.4) in a ratio of 1 gram of feces to 5 mL of PBS and vortexed for 1 minute[1]. Serial dilutions from $5x10^{-1}$ to $5x10^{-4}$ and 0.1 mL aliquots of each dilution were plated on MacConkey agar. Plates were incubated at 37º

C for 20 h and numbers of bacterial colonies were counted and reported in colony forming units per gram of feces (cfu/g)1. For Objective 2, somatic cell counts (SCC) were recorded for all cows in the trial from Farm B from samples sent to the Dairy Herd Improvement (DHI) Cooperative (Columbus, OH). The SCC measurements from the most recent milk tests were used. The average numbers of days since the last test were 2.5 d and 28.5 d for lactating cows and dry cows, respectively. Correlations between SCC and CFU/g of feces were investigated using this data.

Results

Data obtained from plating fecal samples showed no difference between the late-lactation cows and the early-dry cows (P = 0.78; Figure 1). Pearson correlation coefficients were calculated for each variable reported. For all cows in trial, there were no correlations between their coliform bacterial counts and fat-corrected milk (FCM), milk, or SCC (Table 3).

Discussion

The dry period has a significant effect on the incidence of CM in the subsequent lactation. A decrease in coliform bacteria being shed by cows during their transition into the dry period could decrease mastitis-causing bacteria in the environment, thereby decreasing the risk of cows developing CM in the subsequent lactation[7]. Indeed, Hogan et al.,[5] compared the relationship between log gram-negative bacterial counts in the bedding to clinical cases of mastitis and reported a 10% decrease in bacterial counts that would translate to a 0.11 CM cases/305 cow days reduction (or 11 cows out of every 100 on farm). It was anticipated that the change in diet that occurs before and after drying-off would affect coliform bacteria shedding in dairy cattle. The present study failed to show a difference between the coliform bacteria shed before and after drying-off. In fact, coliform bacteria shedding on each farm numerically decreased from lactation

to the dry period, whereas average bacteria shed increased numerically from lactation to the dry period. The variation in bacteria shedding may in part be attributed to the incidences of shedding for each farm. For Farm A, ~16% of cows exhibited no shedding of coliform bacteria. This trend is noted in other studies of dairy cows[1,8].

It is noted that most studies on the effect of grain feeding on bacterial shedding have been conducted with beef feedlot cattle[6,13,14]. Feedlot cattle typically consume a greater (~85% concentrate) grain diet than either lactating (~40 to 60%) or dry cows (~15% concentrate). Diez-Gonzalez et al.[14] published data showing that E. coli counts in beef cattle rose more than 3 logs in the colon when fed 90% grain versus only hay. However, the increase in bacterial counts between 0 and 45% grain was much less defined (less than 1 log). This suggests that the range of grain levels in dairy cow-diets may not reach a level high enough to significantly affect shedding of coliform bacteria.

Further, a study in beef cattle revealed that type of grain is also important, as barley feeding was associated with increased risk of isolating E. coli O157:H7 when compared to corn grain[15]. Thus, varying the sources of grain may also be a potential strategy to combat fecal shedding of bacteria. It is acknowledged that diet formulation is tied to availability of feedstuffs. However, because decreasing fecal shedding may decrease risk of mastitis, feeding a grain that limits high pathogen shedding could be still be economically viable. Scott et al.[12] have already identified that rate of passage of grains does not affect the rate of fecal shedding of E. coli, suggesting that changing the variety of grain, and not just the consistency, may be necessary to alter pathogenic fecal shedding.

Summary

The levels of fecal shedding of coliform bacteria did not change significantly among cows close to drying off and those early in the dry period. Furthermore, SCC was found to not be a reliable indicator for high shedding rates. The levels of fecal shedding (actual cfu/g) decreased amongst the cows on each farm, individually, but not with significance between the two groups. Fecal shedding of bacteria is recognized as a possible cause of CM, however, the change in grain associated with the transition into the dry period did not have a significant effect on levels of shedding in this study. This suggests that, at this time, grain-feeding strategies to decrease fecal shedding rates are not recommended. However, further studies are needed using additional farms with additional cows and following the same cows through the transition from lactation to dry-off.

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Figure

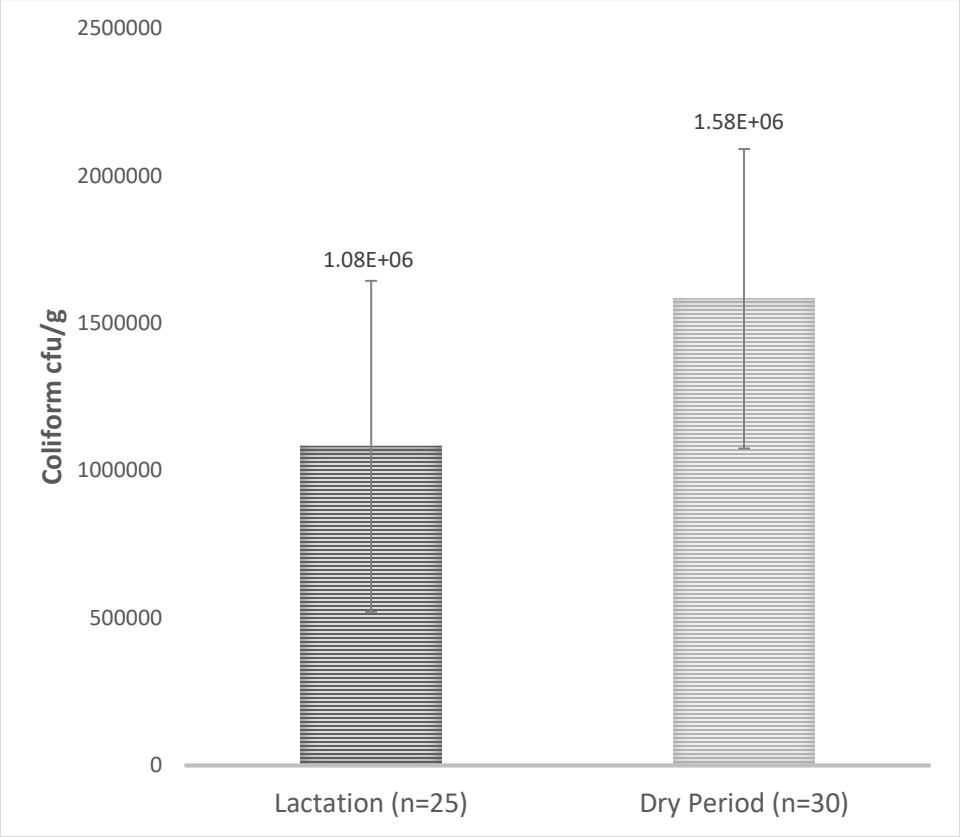

Fig. 1: Comparison of lactating and dry cows in trial in terms of cfu of coliform bacteria per gram of feces (P = 0.78).

Tables

Table 1: Characteristics (% of dietary DM) of diets from each farm[1].

Item	Lactating Cows		Dry Cows	
	Farm A	Farm B	Farm A	Farm B
Corn Silage	41.4	33.54	28.5	41.1
Alfalfa, Grass, or Small Grain Haylage	29.2	6.8	10.7	0.0
Straw	0.0	0.0	33.9	39.4
Concentrates	29.4	59.7	26.9	19.5
NDF	41.6	30.1	49.5	53.0
Starch	18.5	29.9	14.3	15.4

[1]Information on diets was provided by the farm

Table 2: Characteristics of cows used in the study from the two farms[1]. Farm A did not use Dairy Herd Improvement; therefore, there were no milk component data.

Item	Farm A		Farm B		Average	
	Lactation	Dry	Lactation	Dry	Lactation	Dry
Days Since Dry/Days Before Calving						
Average, days	59.8	16.7	65	17.3	61.9	17.0
SD[2], days	2.46	3.61	2.05	3.42	3.44	3.47
Coliforms Shed						
log cfu/g	3.82	3.68	6.09	5.76	4.73	4.79
SD[2]	2.42	2.87	0.55	0.61	2.19	2.27
Milk						
kg/d	19.6	20.0	27.6	23.0	22.6	21.5
SD[2], kg	3.2	4.0	8.3	10.9	7.1	8.2
DIM						
d	349	357	353	366	351	362
SD[2]	52	66	58	62	54	63

[1] Milk data taken 10/12/16 and 10/4/16 for dry cows from Farms A and B, respectively. Milk data taken 11/3/16 and 11/1/16 for lactating cows from A and B, respectively.

[2] Standard deviation

Table 3: Correlation table of variables in experiment.

	Milk	FCM[1]	SCC
Coliforms (log cfu/g)	0.14955	0.2054	-0.06791
p-value	0.2758	0.3246	0.7582
Milk (kg/d)		0.95603	-0.05184
p-value		<0.0001	0.8143
FCM (kg/d) [1]			-0.09422
p-value			0.6689

[1]Fat-corrected milk