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Publication Date

2016-12-01

DOI

10.1016/j.livsci.2016.10.011

Peer reviewed

Livestock Science 194 (2016) 17–22

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

Impacts of dietary forage and crude protein levels on the shedding of *Escherichia coli* O157:H7 and *Listeria* in dairy cattle feces

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ARTICLE INFO

Keywords:
Dairy
Escherichia coli
Feces
Forage
Listeria
Protein

ABSTRACT

The shedding of *Escherichia coli* O157:H7 and *Listeria monocytogenes* in the feces of ruminants and the consequential risk to the public and environmental health is well reported. However, the influence of dietary manipulation on the shedding of fecal bacteria is not well understood. This study was conducted to improve understanding of the relationship between dietary feed composition and shedding of *E. coli* O157:H7 and *Listeria* spp. in dairy feces. Twelve cows were randomly assigned to four treatment diets of two dietary forage levels: low forage (37.4% dry matter, DM) vs. high forage (53.3% of DM) and two dietary crude protein (CP) levels: low protein (15.2% of DM) vs. high protein (18.5% of DM) in a 4×4 replicated Latin square design with four periods each including a 14 d adaptation and 3 d sample collection periods. Generic *E. coli* was detected in some of the feed ingredients, such as cotton seed, alfalfa hay, almond, and $CaCO_3$, while *Listeria* was detected in the alfalfa hay and mineral mix. A significant interaction effect was observed between dietary forage and CP on the presence of fecal *E. coli* O157:H7 (P=0.01) but not with *Listeria*. On average, the greatest *E. coli* O157:H7 level (6.6 $\log_{10}$ CFU/g of feces) was observed from the high forage and high protein diet and the lowest level was 6.1 $\log_{10}$ CFU/g from the low forage and high protein diet. The average *Listeria* shedding rate was within the range of 1.7–2.3 $\log_{10}$ CFU/g among the dietary forage and CP treatments. For the CP treatments, significantly low levels of *Listeria* were observed from cows fed the high protein (0.9–1.6 $\log_{10}$ CFU/g) compared to the low protein (1.3–2.1 $\log_{10}$ CFU/g) diet. Considering temporal fluctuations, no significant diurnal pattern was observed for either *E.coli* O157:H7 or *Listeria*. In addition, no time of sampling over day by dietary forage or CP content interaction on fecal *E.coli* O157:H7 or *Listeria* level was observed. This study showed that diets can influence the shedding of potentially pathogenic bacteria in dairy cow excreta.

1. Introduction

According to the Centers for Disease Control and Prevention (CDC), *Escherichia coli* O157:H7 and *Listeria monocytogenes* are two major foodborne pathogens (CDC, 2014). Both bacteria have been associated with numerous outbreaks in the United States (Ratnam et al., 1988; Nightingale et al., 2004; CDC, 2014). Cattle are reservoirs of *E.coli* O157:H7 as they are found in the lower gastrointestinal tract, specifically the mucosal surface of the rectum (Naylor et al., 2003; Gyles, 2007; Hussein, 2007) and also in feces (Callaway et al., 2003; Berg et al., 2004; Jacob et al., 2008a, 2008b). The detection of *Listeria monocytogenes* was also reported in the feces of ruminants, particularly cattle (Pell, 1997; Pauly et al., 1999). Several factors including feed, water, age of animal, and seasonality can influence the prevalence and shedding of pathogens in ruminants (Caro et al., 1990; Bach et al., 2002; Renter and Sargeant, 2002; Ho et al., 2007). Diet can also influence the physiological condition of the gut and was reported to affect the colonization of *E. coli* O157:H7 and *Listeria* spp., which altered their shedding (Arimi et al., 1997; Buchko et al., 2000; Jacob et al., 2008a). Callaway et al. (2006) reported that *E. coli* O157 (5–20% of samples) and *Listeria* spp. (0–10% of samples) were isolated in fecal samples from 4 feedlots within 45 min of morning feeding.

Even though diet is considered to be an influencing factor, the impact of grain and forage proportions in animal diet on the presence of pathogen in feces is uncertain. Diez-Gonzales et al. (1998) reported that diets containing high grain levels (60–80% rolled corn) favored the growth of acid-resistant *E. coli* compared to diets containing high proportions of hay because of lower ruminal pH. However, Hovde et al.

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http://dx.doi.org/10.1016/j.livsci.2016.10.011
Received 26 August 2016; Received in revised form 26 October 2016; Accepted 29 October 2016
1871-1413/ Published by Elsevier B.V.

(1999) found that the shedding of *E. coli* O157:H7 from hay-fed cattle was longer in duration than from grain-fed cattle. The authors also reported that *E. coli* O157:H7 was equally acid resistant under both the diets. In contrast, Jordan and McEwen (1998) found no differences in fecal *E. coli* levels between two groups of beef cattle fed either low-forage or high-forage diet rations.

Siragusa et al. (1993) reported the presence of *Listeria* spp. (*L. innocua*, *L. monocytogenes*, and *L. welshimeri*) in 9% to 35% of fecal grab samples from healthy feedlot beef cattle in Nebraska. The authors tested 224 individual animals, which received high energy feed consisting of 25% corn-silage, 70% corn, and 5% protein-mineral concentrates. Ryster et al. (1997) isolated *L. monocytogenes* from 2% of corn silage samples (n=129) and 3% of hay silage samples (n=76) indicating the risk of feed-related contamination. Several other studies (Fernandez-Garayzabal et al., 1992; Arimi et al., 1997; Wesley, 1999) have attempted to establish relationships between the excretion of *L. monocytogenes* and silage as feed ingredients in ruminants. Ho et al. (2007) isolated *L. monocytogenes* from 31% of fecal samples from lactating dairy cows fed hay and silage on 24 of 33 (73%) days of sampling. The authors found that 94% of cows excreted *L. monocytogenes* in feces at least once during the study period and it was also detected in 38% of silage samples.

In the United Kingdom, Fenlon et al. (1996) detected *L. monocytogenes* in cattle feces at levels ranging from 2.3 to 20 CFU/g when silage was fed. The authors confirmed that the *L. monocytogenes* serotype and electrophoretic type found in the silage was similar to those detected in the feces. Skovgaard and Morgen (1988) found the presence of *Listeria* spp. in 12% of both fecal and silage samples, which were collected from 7 dairy farms. Considering the existing knowledge gap about the impact of conventional diet regimen on pathogen shedding from dairy cows, this study was designed to evaluate the effect of diet on fecal bacteria in dairy excreta. The objective of the study was to quantify the impacts of dietary forage and crude protein (CP) levels on the excretion and prevalence of *E. coli* O157:H7 and *Listeria* spp. in dairy cattle feces.

2. Materials and methods

2.1. Experimental design and animal feeding

The study was conducted between July and September 2014 at the University of California–Davis dairy facility with all procedures approved by the Institutional Animal Care and Use Committee. Twelve Holstein cows (157 ± 31 d postpartum; mean ± SD) with an average milk production of 39.3 ± 4.4 kg/d and average body weight (BW) of 667 ± 29 kg at the beginning of the study were randomly assigned to 4 treatment diets consisting of 2 forage levels [37.4 (low forage, LF) vs. 53.3% (high forage, HF) of dry matter, DM] and 2 CP levels [15.2 (low protein, LP) vs. 18.5% (high protein, HP) of DM]. The experiment was a 4×4 Latin square design with four 18 d periods. Each period consisted of 14-d adaptation, followed by a 3-d sample collection period. The forage and CP contents in the treatments encompass ranges used in typical lactating dairy cow diets in the USA (Table 1). Cows were fed 105% of previous day intake, 60% of which was offered at 08:00 h and the balance was offered at 20:00 h, which was built on previous experiments (Niu et al., 2014, 2016). Feed refusals (i.e., orts) were removed and weighed before feed delivery in the morning. Individual feed ingredients were sampled at each mixing.

2.2. Sample collection and analysis

Representative samples of the total mixed ration (TMR) diets were collected on day 8, 11, 14, and from day 16–18, while orts (12.5%) were sampled from day 8–18 in each period. Feed and ort samples were composited by period. Feed ingredients were transported to the Extension Lab in the School of Veterinary Medicine for microbial analyses within 24 h of collection. Ort and TMR samples were stored at −20 °C until shipped to Cumberland Valley Analytical Services Inc. (Maugansville, MD) for analysis of DM (135 °C; AOAC, 2000; method 930.15); CP (N ×6.25; AOAC, 2000; method 990.03); neutral detergent fiber (Van Soest et al., 1991); acid detergent fiber (AOAC, 2000; method 973.18); lignin (Goering and Van Soest, 1970); starch [(Hall, 2008) with correction for free glucose]; total ash (535 °C; AOAC, 2000; method 942.05); and minerals. Feed samples were also tested for *E. coli* O157:H7 and *Listeria* spp. to ensure the quality of ingredients.

Table 1
Ingredients and nutrient composition of the experimental diets.

Item	HF[a]		LF	
	HP	LP	HP	LP
Ingredient, % of DM				
Alfalfa hay[b]	53.3	53.3	37.6	37.2
Steam-flaked corn	19.1	27.0	33.7	41.5
Soybean meal	7.5	0.0	12.0	4.3
Whole cottonseed	5.5	5.5	5.5	5.4
Rolled barley	4.2	4.2	4.1	4.1
Almond hulls	2.6	2.6	2.6	2.6
Dry distillers grains	6.2	5.6	2.4	2.5
Mineral and vitamin mix[c]	1.0	1.0	1.0	1.0
$CaCO_3$	0.0	0.1	0.3	0.4
NaCl	0.1	0.1	0.1	0.1
Mineral mix[d]	0.1	0.3	0.1	0.2
Chemical composition,[e]% of DM				
CP	18.7	15.3	18.4	15.1
NDF	31.0	30.8	24.5	24.3
ADF	24.8	24.6	19.2	19.0
Lignin	6.0	6.0	4.9	5.0
Starch	18.5	24.2	28.7	34.3
EE	3.6	3.8	3.6	3.8
Ash	7.4	7.2	7.0	6.7
TDN	68.9	69.1	72.8	73.1
NE_L, Mcal/kg	1.60	1.60	1.69	1.69

[a] (HP=high protein, LP=low protein, HF=high forage, LF=low forage).
[b] Contained 91.5% DM and 17.6% CP, 44.2% NDF, 2.5% starch, and 16.3% tdNDF on a DM basis.
[c] Mineral and vitamin mix compositions (DM basis): 0.49% CP; 0.185% fat; 0.72% NDF; 11.8% Ca; 5.33% P; 9.16% Na; 0.08% K; 0.005% Cl; 4.27% Mg; 2.11% S; 4466.7 mg/kg of Zn; 208.1 mg/kg of Fe; 2666.7 mg/kg of Mn; 666.7 mg/kg of Cu; 58.7 mg/kg of I; 25.1 mg/kg of Co; 22.7 mg/kg of Se; 0.22% Methionine; 0.01% Lysine; 533,874 IU/kg of Vitamin A (retinyl acetate); 184,800 IU/kg of Vitamin D (Activated 7-dehydrocholesterol); 4,180 IU/kg of Vitamin E (dl-α tocopheryl acetate); 58.674 mg/kg of biotin; 933.3 mg/kg of Monensin (Elanco, Greenfield, IN).
[d] Mineral mix (Phosphorus supplement; ICL Performance Products LP, St. Louis, MO) contained: 26% of P; 19.3% of Na; 0.03% of S; 30 mg/kg of F; 50 mg/kg of Fe.
[e] n=3.

2.3. Bacterial enumeration

Fresh fecal samples were collected directly from the rectum at 6 different times during the 3 d (55-h) sample collection (09:00 and 21:00 h on the 1st day; 01:00, 13:00 h on the 2nd day; and 05:00, 17:00 h on the 3rd day) in each period to represent the course of a day. Approximately 300 g of fresh fecal sample from each cow was placed in a sealable plastic bag and stored at 4 °C for no longer than 24 h after collection before being analyzed. In total, 288 fecal samples were collected and analyzed (6 samples/cow/period × 12 cows × 4 periods). The concentrations of *E. coli* O157:H7 and *Listeria* spp. were determined by culture-dependent methods using selective agar media (Hutchison et al., 2004; Berry and Miller, 2005; Biswas et al., 2016). Each fecal sample was homogenized in phosphate-buffered saline solution, serially diluted, and plated in duplicate. For *E. coli* enumeration, MacConkey II agar with sorbitol (BBL, Becton, Dickinson and Company, Sparks, MD, USA) was used. When incubated at 37 °C for 24 h, the *E.coli* O157:H7 produce colorless colonies and other *E. coli*

form sorbitol positive pink colonies. Sorbitol-negative colonies were counted as presumptive *E. coli* O157:H7 without additional confirmation. For enumeration of *Listeria,* Polymyxin-Acriflavin-Lithium chloride-Ceftazidime-Aesculin-Mannitol agar (HiMedia Laboratory, Mumbai, India) was used, where colonies appear as gray-green with a black precipitate after incubation at 35 °C for 24–48 h. Positive control organisms were *E. coli* O157:H7 (ATCC 35150) and *L. monocytogenes* (ATCC BAA-679D-5).

2.4. Statistical analysis

To evaluate the impact of diets on bacteria levels, data were statistically analyzed as a replicated design using PROC MIXED procedure of SAS with repeated measures (SAS Institute Inc., Cary, NC). The full statistical model is given by:

$$Y_{ijklmn} = \mu + S_i + P_j + C_k(S_i) + T_n + F_l + \underset{m}{Pr} + F_l \times \underset{m}{Pr} + F_l \times T_n + \underset{m}{Pr} \times T_n + e_{ijklmn},$$

where Y_{ijklmn} is the response variable of interest, μ is the overall mean, S_i is the random effect of the sequence of treatments assigned on individual cows (i=1–4), P_j is the fixed effect of period (j =1–4), $C_k(S_i)$ is the random effect of cow nested within the sequence (k=1–12), T_n is the fixed effect of sampling time (n=1–6), F_l is the fixed effect of dietary forage level (l=LF or HF), Pr_m is the fixed effect of dietary protein level (m=LP or HP), $F_l \times Pr_m$ is the interaction between forage level and protein level, $F_l \times T_n$ is the interaction between forage level and sampling time, $Pr_m \times T_n$ is the interaction between protein level and sampling time, and e_{ijklmn} is the residual error. The AR(1) and ARH(1) covariance structures (equal spacing) were used for bacteria levels. Covariance structures were selected based on model fit, time was the repeated variable, cow by treatment (dietary protein or forage content) was the subject, and denominator degrees of freedom were adjusted by the Kenward-Rogers method (Niu et al., 2014). The effects of dietary forage and protein levels on fecal bacteria levels at different times of the day were analyzed using a reduced model including time, main effects (forage or protein), and their interactions. The daily average bacteria levels of individual cows were calculated over different times in each period to analyze the main effects and their interactions using a reduced model including main effects (forage or protein), and their interactions. The Student's *t*-test was performed to identify significant differences between the contrasts (α=0.05). Additionally, presence of a diurnal pattern of bacteria levels was tested using a fixed 24-h cosine model as described by Niu et al. (2014). Bacteria levels were log-transformed before performing statistical analysis and “no growth” plates were recorded as 1 CFU/g before log transformation.

3. Results

3.1. Feed bacteria levels

Out of 288 fecal samples tested, 97% and 71% of samples were positive for *E.coli* O157:H7 and *Listeria* spp., respectively. The levels of *E. coli* and *Listeria* in the feed ingredients are presented in Table 2. The presence of generic *E. coli* was observed in cotton seed, alfalfa hay, almond, and $CaCO_3$ samples at a range of 2.4–3.5 $\log_{10}$ CFU/g, while sorbitol-negative colonies on the MacConkey agar, representing *E. coli* O157:H7, were not detected in the feed ingredients. *Listeria* was detected in the alfalfa hay and mineral mix at 2.0 and 1.7 $\log_{10}$ CFU/g, respectively.

3.2. Fecal bacteria levels

The effect of dietary forage and CP contents on the occurrence of *E. coli* O157:H7 and *Listeria* in cow feces are given in the Table 3. There was a forage × CP interaction on the presence of *E. coli* O157:H7 (*P*=0.01). The fecal levels of *E. coli* O157:H7 were 6.6 and 6.5 $\log_{10}$ CFU/g for the HFHP and LFLP diets, whereas the levels were lower at 6.1 and 6.2 $\log_{10}$ CFU/g for the LFHP and HFLP diets, respectively. There was no significant interaction between forage and CP on the *Listeria* levels. However, *Listeria* levels were higher in feces from cows fed LP than HP diets, whereas they were lower in feces from cows fed LF than HF ($P < 0.01$).

The temporal fluctuations of *E.coli* O157:H7 and *Listeria* in cow feces receiving HF or LF, and HP or LP over the course of a day are presented in Figs. 1 and 2, respectively. No significant diurnal pattern was observed for either *E. coli* O157:H7 or *Listeria*. There was no time of sampling by dietary forage or CP content interaction on *E. coli* O157:H7 or *Listeria* levels. The effect on *E. coli* O157:H7 level was not observed for either forage or protein treatments at any time points over the day (Fig. 1). However, fecal *Listeria* levels varied during the day when cows were fed at different dietary CP or forage levels (Fig. 2). Regardless of the CP content in the diet, the fecal level of *Listeria* was higher for HF than LF at 01:00 (*P*=0.04; 1.3 vs. 0.7 $\log_{10}$ CFU/g) and 13:00 h (*P*=0.01; 1.9 vs. 1.2 $\log_{10}$ CFU/g), and tended to be higher at 17:00 h (*P*=0.07; Fig. 2). In terms of dietary CP effect, *Listeria* in feces was found to be higher for LP than HP at 13:00 ($P < 0.01$; 1.9 vs. 1.1 $\log_{10}$ CFU/g) and 17:00 h (*P*=0.01; 1.8 vs. 1.1 $\log_{10}$ CFU/g), and tended to be higher at 05:00 and 09:00 h ($P < 0.10$). As mentioned previously, a diurnal pattern was not observed for either *E. coli* or *Listeria*, but the levels generally showed a rising trend towards the

Table 2
Quantitation of bacteria in the feed ingredients.

Ingredients	*E. coli*[a] ($\log_{10}$ CFU/g)	*Listeria* spp. ($\log_{10}$ CFU/g)
Stream-flaked corn	ND[b]	ND
Soybean meal	ND	ND
Whole cottonseed	3.5	ND
Mineral mix	ND	1.7
Dry distillers grains	ND	ND
$CaCO_3$	2.8	ND
Alfalfa hay	3.5	2.0
NaCl	ND	ND
Rolled barley	ND	ND
Almond hulls	2.4	ND

[a] All feed ingredients were negative for *E.coli* O157:H7.
[b] ND=not detected.

Table 3
Effect of dietary forage and crude protein content on *E. coli* O157:H7 and *Listeria* spp. levels in dairy cows feces.

Item	Treatment LSM (n=12)[a]				SE[b]	*P*-value		
	HF		LF					
	HP	LP	HP	LP		Forage	Protein	Forage × Protein
E. coli O157:H7 ($\log_{10}$ CFU/g)	6.6[a]	6.2[ab]	6.1[b]	6.5[a]	0.2	0.77	0.91	0.01
Listeria spp. ($\log_{10}$ CFU/g)	1.9[a]	2.3[b]	1.7[a]	2.0[a]	0.1	< 0.01	< 0.01	0.35

[a] HF=high forage diet, LF=low forage diet, HP=high crude protein diet, LP=low crude protein diet; different letters in the same row indicate the significant difference of contrasts using Student's *t*-tests.
[b] SE=standard error.

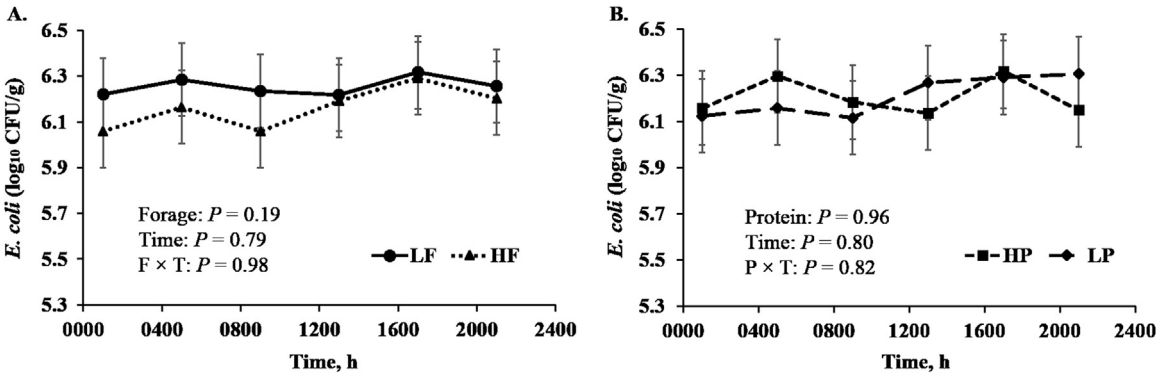

Fig. 1. Change in *E. coli* O157:H7 levels over the course of a day considering (A) two forage levels (HF=high forage, LF=low forage) and (B) two crude protein levels (HP=high protein, LP=low protein). Main effect of two factors (Forage and Protein), effect of time, and their interactions (F x T and P x T) are shown within each panel.

middle of the day and then a slight decreasing trend afterwards.

4. Discussion

Forage and protein levels can have considerable influence on the presence of microbial populations found in the rumen and gastrointestinal tract of cattle (Gouws and Kistner, 1965; Russell, 1984). In the current study, CP contributed around 15–19% of the diet DM, whereas carbohydrates and minerals made up the remainder. Structural and non-structural carbohydrates are the primary source of energy and control the microbial growth and other supportive function in rumen. Fermentation of carbohydrates by rumen microbes generates volatile fatty acids (VFAs), which are the main source of energy for the cows. Type of dietary carbohydrate determines fermentation rate and type of VFA having a significant impact on rumen pH. Low forage diets contain greater amounts of non-structural carbohydrates such as starch, which are more rapidly fermented in the rumen compared to structural carbohydrate such as cellulose. Fermentation of high amounts of starch in the rumen and the hind gut can reduce the pH to extents that can negatively affect the growth and survival of microorganisms (Russell et al., 2000; Fox et al., 2007).

Recent studies indicated that diet can greatly influence community structure of fecal microbiota of cattle (Callaway et al., 2010; Shanks et al., 2011; Rice et al., 2012). Callaway et al. (2010) conducted a study where feedlot cattle were randomly assigned 3 diets, where 0, 25, or 50% of the grain supplement was replaced with dried distillers grains (DDG). The authors found that rumen and fecal bacterial populations were different when animals were fed DDG compared with controls. Moreover, they observed low rumen pH in cattle fed with diets containing 50% compared with 0% DDG. Shanks et al. (2011) profiled the fecal microbial communities of cattle from 6 different feeding operations (5 animals per operation). Sequence-based clustering and taxonomic analyses indicated greater variability in fecal microbial communities between operations than within an operation. In addition, they found that bacterial community composition correlated significantly with fecal starch levels. This was largely reflected in changes in the Bacteroidetes, Proteobacteria, and Firmicutes populations. Rice et al. (2012) assigned 20 cattle to 5 steam flaked corn-based diets (n=4) each with 0, 5, 10, or 15% (DM basis) of sorghum wet distillers grains, or 10% wet corn distillers grains and collected fecal grab samples from individual animals for fecal microbiome analyses. They observed a total of 24 phyla among the samples revealing considerable variability among animals. However, six high abundance phyla (Firmicutes > Bacteroidetes > Proteobacteria > Tenericutes > Nitrospirae > Fusobacteria) were observed in all animals regardless of dietary treatment, whereas four low abundance phyla significantly responded to dietary treatments. In line with the previous studies showing notable effects of diet composition on fecal microbiome of cattle, there was a significant interaction between dietary forage and CP contents on the prevalence of fecal *E. coli* O157:H7 of dairy cows, in the present study. The changes in dietary forage and CP composition may affect the ruminal digestibility (Broderick, 2003) as well as fermentation efficiency and consequently change the ruminal pH and microbial profile (Esdale and Satter, 1972; Mackie and Gilchrist, 1979; Shriver et al., 1986).

Dargatz et al. (1997) collected fecal samples from cattle pens in a total of 100 feedlots in 13 states and found that there was an increasing likelihood of a pen having *E. coli* O157 while feeding barley, a major source of starch compared to soybean meal, a major source of protein. However, the results of our study showed that dietary CP content interacted with dietary forage content in the shedding of *E. coli* O157:H7. For cows receiving a high amount of forage (HF), fecal *E. coli* O157:H7 level tended to increase when the CP content was high (P=0.06), whereas for cows receiving less forage (LF) fecal *E. coli*

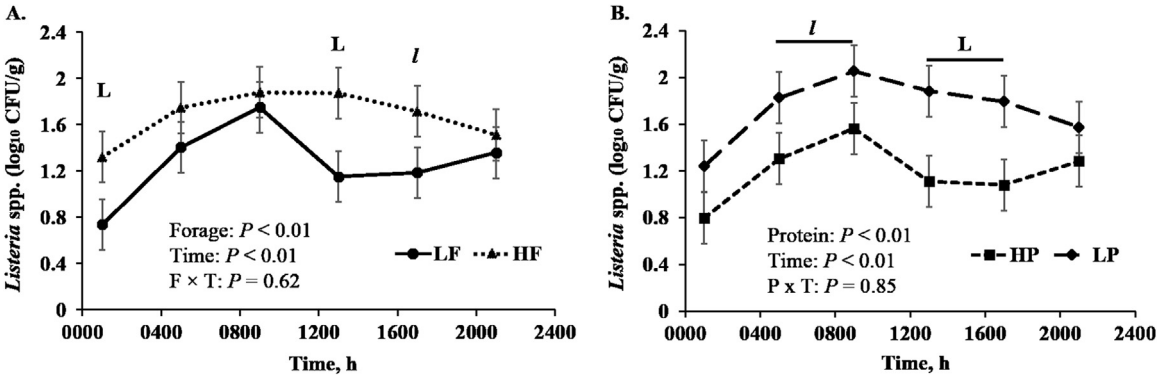

Fig. 2. : Change in *Listeria* spp. levels over the course of a day considering (A) two forage levels (HF=high forage, LF=low forage) and (B) two crude protein levels (HP = high protein, LP = low protein). Main effect of two factors (Forage and Protein), effect of time, and their interactions (F x T and P x T) are shown within each panel. Effects of two factors (HF vs. LF and HP vs. LP) were tested (^{L} =P < 0.05, ^{l} =P < 0.1) for every 4 h.

O157:H7 level tended to decrease when CP content was high (P=0.08). A review of the existing literature showed that our study design (i.e., allowing for testing interactions between dietary nutrients on pathogen shedding of cattle) is unreported. Berg et al. (2004) tested fecal *E. coli* O157:H7 levels in cattle fed a finishing (low-forage) diet supplemented with barley or corn, respectively with high and low CP contents. Contrary to our observations regarding LF diets, they observed that the prevalence of fecal *E. coli* O157:H7 in high-protein barley-fed cattle was greater than low-protein corn-fed cattle. They also reported that corn-fed cattle had lower average fecal pH values (5.9) than did barley-fed cattle (6.5) depending on the differential fermentation rates of starch in corn and barley. Hence, it is difficult to draw a conclusion on the primary cause for the differences in fecal *E. coli* O157:H7 prevalence between two diets as dietary CP content and rate of carbohydrate fermentation were confounded. Similar confounding results may occur even across the present diets since the steam-flaked corn is known to have a strong influence on rumen pH with varying CP contents. Nonetheless, availability of N is important for the growth of microorganisms including *E. coli*. The greater prevalence of *E. coli* O157:H7 for HP than LP in cows fed high-forage diets generally associated with healthy rumen and perhaps hindgut pH indicate that N availability was critical for the growth of *E. coli* O157:H7. On the other hand, the low fecal *E. coli* O157:H7 prevalence for HP in cows receiving low-forage diet is unexpected as N availability would be further critical for growth in the harsh environment with a lower pH created by the high grain diet.

In the present study, forage content in the diet did not have significant impact on fecal *E. coli* O157:H7 levels. Conversely, some previous studies have shown that changes in forage levels (hay content) may impact the *E. coli* population in feces (Keen et al., 1999, Diez-Gonzalez et al., 1998). When Diez-Gonzalez et al. (1998) changed the beef cattle ration from a 90% grain diet (mostly rolled corn) to 100% hay diet; there was a 5-fold decrease in the generic *E. coli* population in fecal samples. Keen et al. (1999) divided 200 grain-fed cattle into two groups and one group was fed the same grain and the other group was abruptly switched to hay. They found that 52% of the grain-fed cattle remained *E. coli* O157:H7 positive, but only 18% of the hay-fed cattle continued to shed *E. coli* O157:H7. However, the studies showing a significant effect of dietary forage content on the *E. coli* O157:H7 prevalence changed the forage content in the diet more abruptly (mostly by 100%) than in the present study (by about 40%). Therefore, notable changes in fecal *E. coli* O157:H7 levels appear to be associated with larger changes in dietary forage content, although such changes might have significantly adverse impact on production (e.g., milk fat depression) and well-being (e.g., lameness) of dairy cows.

Unlike *E. coli* O157:H7, only a few studies are available that evaluated the impact of diet on *Listeria* spp. in dairy feces. In a study by Fenlon et al. (1996), about 30% of cattle in the herd shed *L. monocytogenes* after being fed silage. Ho et al. (2007) found *L. monocytogenes* in 38% of the silage samples, with 94% of cows excreting *L. monocytogenes* in feces at least once during the study. Nonetheless, no study has specifically studied the impact of diet composition on fecal shedding of *Listeria* by cattle. In the present study, the likely reason why the cows excreted *Listeria* was because the alfalfa hay contained *Listeria* and it was the largest portion of the total mixed ration. We observed that the basic diet composition characterized by forage and CP contents (with no significant interaction between the two) to influence *Listeria* shedding in feces. Specifically, high forage or low protein produced the greatest *Listeria* levels on average when compared to low forage or high protein, respectively. Furthermore, we found that *Listeria* levels in feces varied temporally, but did not demonstrate of diurnal pattern, and were greatest at 8:00 h regardless of forage or protein level. This result is supported by Ho et al. (2007), who found that the prevalence of *L. monocytogenes* during fecal shedding fluctuated considerably over time.

5. Conclusion

In summary, our results indicate that forage and CP levels in animal feed have the potential to influence the fecal shedding of pathogens in dairy cattle. Both *E. coli* O157:H7 and *Listeria* spp. are common fecal bacteria associated with ruminant animals. They are also directly linked with the human health risks through the food chain. Thus, a change in dietary composition might be a possible way to reduce the release of these bacteria to the environment. Although it may not be possible to eliminate pathogenic bacteria from dairy feces, efforts should put toward best manure management practices. Before that, optimal diet combinations should be investigated to reduce/control their excretion, while considering the ultimate output (quality of meat/dairy) and economic significance. Additional studies are required to identify other factors that can influence the microbiome of the gastrointestinal tract of dairy cattle under different diet regimens.

Acknowledgments

This research was funded through a cooperative agreement (no. 58-5368-4-004) with USDA-ARS Northwest Irrigation and Soils Research Laboratory (Kimberly, ID). The authors also acknowledge the assistance of Tekeste Tewoldebrhan, Jasmin Grey, Anna Naranjo, and Doug Gisi (University of California-Davis, Davis, CA).

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