SdiA Aids Enterohemorrhagic *Escherichia coli* Carriage by Cattle Fed a Forage or Grain Diet

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Enterohemorrhagic *Escherichia coli* (EHEC) causes hemorrhagic colitis and life-threatening complications. The main reservoirs for EHEC are healthy ruminants. We reported that SdiA senses acyl homoserine lactones (AHLs) in the bovine rumen to activate expression of the glutamate acid resistance (*gad*) genes priming EHEC's acid resistance before they pass into the acidic abomasum. Conversely, SdiA represses expression of the locus of enterocyte effacement (LEE) genes, whose expression is not required for bacterial survival in the rumen but is necessary for efficient colonization at the rectoanal junction (RAJ) mucosa. Our previous studies show that SdiA-dependent regulation was necessary for efficient EHEC colonization of cattle fed a grain diet. Here, we compared the SdiA role in EHEC colonization of cattle fed a forage hay diet. We detected AHLs in the rumen of cattle fed a hay diet, and these AHLs activated *gad* gene expression in an SdiA-dependent manner. The rumen fluid and fecal samples from hay-fed cattle were near neutrality, while the same digesta samples from grain-fed animals were acidic. Cattle fed either grain or hay and challenged with EHEC orally carried the bacteria similarly. EHEC was cleared from the rumen within days and from the RAJ mucosa after approximately one month. In competition trials, where animals were challenged with both wild-type and SdiA deletion mutant bacteria, diet did not affect the outcome that the wild-type strain was better able to persist and colonize. However, the wild-type strain had a greater advantage over the SdiA deletion mutant at the RAJ mucosa among cattle fed the grain diet.

Enterohemorrhagic *Escherichia coli* (EHEC) is responsible for major outbreaks and sporadic cases of hemorrhagic colitis and hemolytic uremic syndrome (HUS) throughout the world. Infection can begin after exposure to as few as 50 CFU. EHEC colonizes the large intestine of humans and forms attaching and effacing (AE) lesions on intestinal epithelial cells (1). The AE lesion is characterized by the destruction of the microvilli and rearrangement of the cytoskeleton to form pedestal-like structures that cup the bacteria (2–4). The genes involved in the formation of the AE lesion are encoded within a chromosomal pathogenicity island named the locus of enterocyte effacement (LEE) (5). The LEE region contains five major operons, *LEE1*, *LEE2*, *LEE3*, *LEE5*, and *LEE4* (6–8), that encode a type III secretion system (TTSS) (9), an adhesin (intimin) (10) and its receptor (Tir) (11), and effector proteins, which together with Tir are translocated into the epithelial cell through the bacterial TTSS (12–16) (Fig. 1). The LEE-encoded TTSS also translocates effector proteins encoded outside the LEE region (NleA, B, C, D, E, and F and EspFu/TccP); these other effectors are also important for virulence and pedestal formation (17–23). Additionally, EHEC also requires the LEE to efficiently colonize the rectoanal junction (RAJ) mucosa of healthy cattle, the bacteria's main reservoir (24, 25).

Several studies have also shown that other genes beyond the LEE are needed for EHEC to efficiently colonize cattle. The glutamate decarboxylase (*gad*) acid resistance system is important for EHEC's survival and colonization of the bovine gastrointestinal tract (26). Our previous study shows that duodenal digesta from animals fed either a grain diet or a hay diet was acidic, with pH values ranging from 4.33 to 4.75 (27). Acid resistance is important for bacterial survival in the bovine gastrointestinal tract. We reported that SdiA senses acyl homoserine lactones (AHLs) in the rumen to activate expression of the glutamate acid resistance (*gad*) genes to induce EHEC's acid resistance before passing into the

acidic abomasum stomach. A signature-tagged mutagenesis (STM) study also shows that several genes, including *sdiA*, play a role in the colonization of the bovine intestine (28). Our previous competition studies show that an EHEC *sdiA* mutant is defective for survival within the rumen and colonization of the RAJ mucosa among cattle fed a grain diet (29). SdiA is a LuxR-type transcription factor. LuxR transcription factors are part of the LuxR/I-type quorum sensing systems found in several Gram-negative bacteria. In these systems, the signaling molecule is usually an AHL. The LuxR/I system was the first to be described in *Vibrio fischeri* (30). The luciferase operon in *Vibrio fischeri* is regulated by two proteins, LuxI, which is responsible for the production of the AHL autoinducer, and LuxR, which is activated by this autoinducer to increase transcription of the luciferase operon (31, 32). Since this initial description, homologues of LuxR–LuxI have been identified in other bacteria and shown to regulate the transcription of several genes involved in a variety of phenotypes, including biofilm formation, regulation of several virulence genes, and production of pigments, among others (33–35). The LuxI-type proteins are the AHL synthases. AHLs have a conserved homoserine lactone ring connected through an amide bond to a variable acyl chain. Acyl

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FIG 1 Schematic regulatory model of SdiA virulence gene regulation in EHEC. SdiA directly repressed expression of *ler* (solid line), encoding the master activator of all LEE genes. SdiA activates *gad* expression indirectly (dashed line).

chains vary among 4 to 18 carbons, and the third position may or may not be modified (carbonyl group, hydroxyl, or fully reduced). Different acyl chains ensure that different AHLs will be recognized by different LuxR-type proteins. The substrates used by LuxI-type proteins for AHL synthesis are S-adenosyl methionine (SAM) to synthesize the homoserine lactone ring, and the acyl chains are acquired from lipid metabolism, carried by various acyl carrier proteins (ACP) (36–38). The LuxR-type proteins usually recognize a specific AHL. Due to this feature, this signaling system has been associated primarily with intraspecies signaling. However, there are examples of LuxR-type proteins (such as SdiA) that recognize more than one AHL and are involved primarily in interspecies signaling (39–42).

AHLs have been detected in the rumen of cattle (29, 43, 44) but not in the other gastrointestinal (GI) tract compartments of these animals (29). Through SdiA, rumen AHLs repress transcription of the LEE and activate expression of the *gad* genes (Fig. 1), promoting EHEC colonization in cattle (29). It is assumed that these AHLs are being produced by the rumen microbiota. Our previous studies concerning the role of SdiA in EHEC cattle colonization were performed using animals fed a grain diet (29). It is known that dietary conditions can have profound effects in the composition of the GI microbiota (45). It is important to determine the role of SdiA in bacterial colonization of cattle fed different diets. Here, we showed that cattle fed a forage hay diet also harbor AHLs in their rumen and regulate EHEC SdiA-dependent gene expression in a fashion similar to that of cattle fed grain. In both grain and hay diets, SdiA was important for survival within the rumen and colonization of the RAJ mucosa, although SdiA's role in RAJ mucosa colonization seems to be more pronounced in animals fed a grain diet.

MATERIALS AND METHODS

Strains and plasmids. EHEC strain 86-24 was isolated from a patient with bloody diarrhea (46). The *sdiA* mutant strain DH1 and complemented strain DH2 (*sdiA* gene cloned into pACYC177) have been previously reported (29). *E. coli* strains were grown aerobically in Luria-Bertani (LB) medium or Dulbecco's modified Eagle's medium (DMEM) (Invitrogen) at 37°C unless otherwise stated. Antibiotics were added at the following concentrations: 100 µg ml⁻¹ ampicillin, 30 µg ml⁻¹ chloramphenicol, and 50 µg ml⁻¹ kanamycin.

Quantitative real-time RT-PCR. RNA was extracted from strains 86-24, DH1 (86-24 *sdiA* mutant), and DH2 (mutant complemented with

sdiA), grown in DMEM at 37°C to an optical density at 600 nm (OD₆₀₀) of 1.0 in the absence or presence of rumen-extracted AHLs, where 5 µl of the evaporated rumen extract was added to DMEM. RNA from three biological replicate cultures of each strain was extracted at the late exponential growth phase (OD₆₀₀ of 1.0) using the RiboPure bacteria RNA isolation kit (Ambion) according to the manufacturer's guidelines. The LerTf/LertrR and GadXrtF/GadXrtR primers used in the real-time assays were designed using Primer Express version 1.5 (Applied Biosystems) and have been previously reported (29). Real-time reverse transcription-PCR (RT-PCR) was performed in a one-step reaction using an ABI 7500 sequence detection system (Applied Biosystems). For each 20-µl reaction mixture, 10 µl 2× SYBR master mix, 0.1 µl Multi-Scribe reverse transcriptase (Applied Biosystems), and 0.1 µl RNase inhibitor (Applied Biosystems) were added. Amplification efficiency of each of the primer pairs was verified using standard curves of known RNA concentrations. Melting-curve analysis was used to ensure template specificity by heating products to 95°C for 15 s, followed by cooling to 60°C and heating to 95°C while monitoring fluorescence. Once the amplification efficiency and template specificity were determined for each primer pair, relative quantification analysis was used to analyze the unknown samples using the following conditions for cDNA generation and amplification: 1 cycle at 48°C for 30 min, 1 cycle at 95°C for 10 min, and 40 cycles at 95°C for 15 s and 60°C for 1 min. The RNA polymerase subunit A gene (*rpoA*) was used as the endogenous control. Real-time RT-PCR primers for the LEE genes and *rpoA* have been previously described (47).

AHL extraction from rumen and detection of extracted AHL through TLC. AHL extraction and detection were performed as previously described (48). Briefly, 30 ml of rumen fluid was extracted 2 times with dichloromethane and concentrated to 5 µl using a rotary evaporator. For analytical thin-layer chromatography (TLC), 5 µl of concentrated extract was applied to C₁₈ reverse-phase TLC plates (200-µM layer; Whatman), and the chromatograms were developed with methanol-water (70:30, vol/vol). After development, the dried plates were overlaid with a culture of the *Agrobacterium tumefaciens* *traI::lacZ* (49) indicator strain and 80 µg/ml 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) for 16 h at 30°C. For preparative plates, 50 ml of GI content was extracted 2 times with dichloromethane and concentrated to 5 µl and the plates were spotted, but no chromatography was performed before overlay. The positive control, N-hexanoyl-L-homoserine lactone (C6-AHL) (402 pmol), was also included. For the alkali-acid treatment, 5 µl of rumen extract in dichloromethane was evaporated and 100 µl of 0.1 M NH₄OH was added. After 3 h, 50 µl of the alkali-treated sample was evaporated, while 50 µl of 1 M HCl was added to the remaining 50 µl of alkali-treated sample. After 3 h, the alkali-acid sample was evaporated. This process was repeated with 15 pmol of C8-oxo-AHL. A total of 5 µl of dichloromethane was added back to all samples, and analytical TLC was performed.

Animals and diet. All protocols were approved by the Institutional Animal Care and Use and Biosafety Committees at the University of Idaho. Eight 15-month-old Charolais heifers were surgically fitted with ruminal cannulae and identified with ear tags. The animals were housed in an isolation barn in separate containment pens and had free access to salt blocks and water. The experiment was divided into two parts based on different diet regimens. Survival and persistence of the EHEC *sdiA* mutant (DH1) was tested in the same cattle fed a high-grain diet (previously published [29]) or a hay diet. Cattle were acclimated for 1 month to either a grain diet containing 90% corn and 10% alfalfa hay (29) or, subsequently, after the first experiment was completed and cattle were no longer shedding EHEC, to a forage-based diet containing 90% alfalfa hay and 10% grain prior to bacterial challenge. The forage-based diet is referred to throughout as the hay diet. Rumen fluid and fecal pH values were measured using a Φ45 pH meter (Beckmann Instruments, Fullerton, CA).

Bacterial strains and challenge method. EHEC strain 86-24 (*stx* deletion and streptomycin resistance) and its derivative *sdiA* mutant (streptomycin and chloramphenicol resistance, strain DH1) were used in this

FIG 2 AHLs are also present within the rumen of hay-fed cattle. (A) TLC from AHLs extracted from 30 ml (evaporated to 10 μ l, which was added to the TLC) of rumen fluid and the positive control, oxo-C6-HSL. (B) Quantitative RT-PCR (qRT-PCR) of *ler* in the absence (DCM, dichloromethane) and presence of rumen AHLs.

trial. There was no difference in the growth rates of the parental and the *sdiA* mutant strains in LB broth. The parental and mutant bacteria were grown separately for 18 h at 37°C in LB broth with aeration, adjusted to the same OD₆₀₀, and mixed in a 1:1 ratio prior to cattle challenge. Each animal received a single dose of 10¹⁰ CFU containing equal numbers of the parental and *sdiA* mutant strains placed directly into the rumen.

Sampling and culture. Rumen fluid was sampled from each heifer prior to bacterial challenge and thereafter as indicated in Results. Approximately 30 ml of rumen fluid was obtained from rumen samples collected aseptically with a sterile gloved hand from each heifer. Rumen fluid was filtered through three layers of cheese cloth into a sterile 50-ml tube and held on ice for transport to the laboratory for culture.

Rectoanal junction mucosal swab (RAMS) samples were obtained as previously described (50, 51) prior to bacterial challenge and on the days indicated. Briefly, a sterile foam swab was inserted into the rectum of each steer and moved 10 times in a circular motion around the RAJ mucosa. Each swab was placed in 3 ml Trypticase soy broth (TSB) and held on ice for transport to the laboratory.

E. coli O157 in the bovine samples were cultured and identified using MacConkey sorbitol agar (Difco, Sparks, MD) plates supplemented with 50 ng/ml cefixime (Sigma, St. Louis, MO), 2.5 μ g/ml potassium tellurite (Sigma), 40 μ g/ml vancomycin (Sigma), and 0.1 mg/ml 4-methylumbelliferyl- β -D-glucuronide (MUG) (Biosynth, Switzerland) (SMAC-CTVM). TSB was used for enrichment culture, as in our previous publications (50, 51). *E. coli* O157:H7 colonies were identified as sorbitol negative and MUG negative and confirmed to be of the O157 serotype by latex agglutination.

The numerical data obtained by this procedure are noted as CFU/ml from rumen fluid or CFU/swab from RAMS. Samples negative by direct culture were enriched by incubation at 37°C overnight with aeration, and serial dilutions were plated on SMAC-CTVM. Positive enrichment cultures were given a numerical value of <10 CFU.

After isolation on SMAC-CTMV, the ratio of parental wild-type (WT) to mutant CFU in samples was determined by antibiotic resistance using SMAC-streptomycin and SMAC-chloramphenicol agar. At least 20 separate isolates from each sample were characterized. The competitive index for isolates from each heifer at each sampling time was determined by dividing the number of mutant isolates by the number of WT isolates. Output and input ratios were compared using a Mann-Whitney test. A *P* value of <0.05 was considered significant.

RESULTS

AHLs are present in the rumen of cattle fed a hay diet. Our previous studies with cattle fed a grain diet show that AHLs are present in rumen fluid and that these AHLs activate transcription of the *gad* genes in an SdiA-dependent fashion (29). Because changes in diet can profoundly affect the composition of the GI microbiota (45, 52), which we assume is responsible for the AHL produced within the ruminant rumen, we wanted to assess whether AHLs are present in the rumen of the same heifers fed a forage hay diet. Figure 2A shows the presence of AHLs in the rumen, and the AHLs extracted from the eight different rumen samples repressed ex-

FIG 3 Rumen AHLs activate *gad* expression in an SdiA-dependent manner. qRT-PCR of the *gadX* gene in the WT, *sdiA* mutant, and complemented strains in the absence and presence of rumen AHLs.

TABLE 1 pH values of rumen fluid and feces of cattle fed hay

Heifer	pH value ^a at postdose day:			
	7		28	
	Rumen fluid	Feces	Rumen fluid	Feces
V1	7.22	7.15	7.12	7.28
V2	6.95	7.03	7.24	7.37
V3	7.17	7.28	7.09	7.14
V4	7.29	7.19	7.33	7.43
V5	7.07	7.43	7.07	7.11
V6	7.36	7.73	7.34	7.25
V7	7.09	7.07	7.25	7.41
V8	7.03	7.23	6.98	7.33
Mean	7.15	7.26	7.18	7.29
SE	0.15	0.23	0.13	0.12

^a pH values of rumen and fecal samples of grain-fed heifer have been previously published.

TABLE 2 Number of *E. coli* O157 CFU in rumen fluid of hay-fed heifers after oral dose

Heifer ^a	<i>E. coli</i> O157:H7 CFU/ml of rumen fluid after oral dose ^b					
	1 h	4 h	Day 1	Day 2	Day 3	Day 7
V1	8.60×10^5	5.10×10^3	1.55×10^3	3.10×10^1	N	N
V2	2.47×10^5	3.70×10^4	7.90×10^2	1.00×10^2	N	N
V3	1.13×10^6	2.70×10^4	1.12×10^3	5.00×10^1	5.00×10^1	W+M+
V4	7.50×10^5	2.56×10^4	4.00×10^2	1.80×10^2	1.60×10^2	W+M+
V5	2.43×10^6	9.50×10^3	2.60×10^2	3.00×10^1	W+M+	N
V6	4.40×10^5	2.80×10^4	1.30×10^3	2.00×10^1	W+M+	W+M+
V7	1.75×10^6	4.40×10^3	7.10×10^2	7.00×10^1	N	N
V8	9.40×10^6	3.50×10^4	2.60×10^2	2.00×10^1	9.00×10^1	W+M+

^a Eight 15-month-old Charolais heifers were challenged with an oral dose of 10^{10} CFU containing equal numbers of the parental strain and the *sdiA* mutant. Samples were taken (hour or day) postdose.

^b N, sample was negative by both direct and enrichment culture methods; W+M+, sample was positive only by enrichment culture and contained both the parental and the mutant strains. Bacterial culture data on grain-fed heifer have been previously published.

pression of *ler* in WT EHEC (Fig. 2B). Rumen-extracted AHLs also activated expression of *gadX* (the major regulator of the *gad* genes) in WT EHEC but not in the *sdiA* mutant. This regulation was rescued with gene complementation (Fig. 3).

pH of rumen and feces. The grain diet, compared to the hay diet, resulted in more acidic conditions in the rumen and feces. The mean pH values of rumen fluid and feces from the heifers fed the grain diet were 5.98 and 6.03 (of note, the data of grain-fed cattle have been previously published [29]), respectively, whereas the mean pH values of rumen fluid and feces from the heifers fed the hay diet were 7.16 and 7.27, respectively (Table 1).

EHEC persistence in the rumen and at the RAJ mucosa in the hay-fed heifers compared to that in the grain-fed heifers. All eight heifers were EHEC O157:H7 culture negative before bacterial challenge. Each received 10^{10} CFU containing equal numbers of the parental and *sdiA* deletion mutant. Rumen fluid samples from all heifers were culture positive for *E. coli* O157:H7 at 1 h, 4 h, 1 day, and 2 days postchallenge (Table 2). By day 3, some heifers were rumen fluid culture negative for EHEC. When fed the grain diet, all animals were rumen culture negative by day 8 (of note, the data of grain fed cattle have been previously published [29]). When fed the hay diet, all animals were rumen culture negative by day 11. Among the grain-fed heifers, only one was culture positive by enrichment culture on day 19, and all heifers were negative for EHEC thereafter (29). However, three animals among the eight

hay-fed heifers remained culture positive for the bacteria on day 24, and one was culture positive for 31 days (Table 3).

The parental wild-type strain outcompeted the *sdiA* deletion mutant in the rumen and at the RAJ mucosa among heifers fed either the grain or hay diet. Fewer *sdiA* mutant CFU than wild-type CFU were recovered from rumen fluid among all heifers beginning 1 h postchallenge (Fig. 4) (of note, the data of grain-fed cattle have been previously published [29]). The advantage of the WT over the *sdiA* mutant in the rumen was similar in both grain- and hay-fed animals. Similarly, fewer *sdiA* mutant CFU than wild-type CFU were recovered from the RAMS samples of all heifers, except from one sample on day 3 (Fig. 5). However, in the RAJ, even though the WT had a competitive advantage in animals in both diets, this advantage was more pronounced in cattle on a grain diet (28) (Fig. 4).

DISCUSSION

Colonization of cattle herds by EHEC is linked to EHEC outbreaks, either directly through contaminated beef products or contact with animals or indirectly by environmental distribution of the pathogen in manure that then contaminates water or other food products. The effect of diet on cattle carriage of EHEC has been controversial and confounded by studies with generic *E. coli* that try to extrapolate results to include *E. coli* O157:H7. Many studies show that a grain diet supports higher concentrations of

TABLE 3 Number of *E. coli* O157:H7 CFU recovered from RAMS samples from hay-fed heifers

Heifer ^a	<i>E. coli</i> O157:H7 CFU/swab postdose day ^b							
	1	2	3	7	11	15	21	24
V1	2.43×10^4	9.60×10^3	8.10×10^4	N	N	N	N	N
V2	2.82×10^4	1.20×10^4	6.60×10^3	7.80×10^2	8.10×10^3	5.40×10^3	2.70×10^3	1.08×10^4
V3	8.40×10^4	7.20×10^3	3.00×10^3	2.46×10^4	8.40×10^3	1.80×10^4	1.70×10^3	3.75×10^4
V4	5.40×10^4	1.90×10^4	2.70×10^4	5.40×10^4	1.20×10^2	N	N	N
V5	2.91×10^3	2.40×10^3	9.30×10^2	W+M+	W+M+	6.00×10^1	N	N
V6	3.90×10^5	7.20×10^3	1.56×10^3	4.40×10^4	9.00×10^1	6.00×10^1	3.00×10^1	W+M–
V7	1.80×10^4	5.40×10^3	5.40×10^3	1.20×10^3	W–M+	N	N	N
V8	1.65×10^5	2.04×10^4	2.82×10^4	3.50×10^3	9.00×10^1	3.00×10^1	N	N

^a Eight 15-month-old Charolais heifers were challenged with an oral dose of 10^{10} CFU containing equal numbers of the parental strain and the *sdiA* mutant. RAMS were taken days postchallenge.

^b N, sample was negative by both direct and enrichment culture; W+M+, sample was positive only by enrichment culture and contained both the wild type and the mutant; W+M–, sample was positive by enrichment culture only and only the parental strain was isolated; W–M+, sample was positive by enrichment culture only and only the mutant strain was isolated. Grain-fed heifer data have been previously published.

FIG 4 Competition index of the EHEC WT and *sdiA* mutant in hay-fed cattle. Eight 15-month-old Charolais heifers were fed a grain diet or a hay diet and challenged with equal numbers of the parental wild type and the *sdiA* mutant placed directly into the rumen. The ratio of mutant to WT isolates was determined in the rumen fluid. Competitive index (CI) values equal to 1 indicate no difference between strains, values of <1 indicate that the wild type survives/colonizes better than the mutant, and values of >1 indicate that the mutant survives/colonizes better than the wild type. The horizontal line indicates the mean CI value.

generic *E. coli* in cattle (27, 53–55). However, in experimental challenge studies that specifically measure *E. coli* O157:H7, this is not the case. Forage diets show either no differences in concentration and duration of *E. coli* O157:H7 shedding or higher concentrations and increased durations of *E. coli* O157:H7 shedding (27, 56–59). This study, using the same eight animals and comparing their carriage of EHEC while fed a grain (29) or hay diet, supported earlier work showing that cattle fed a forage-based diet are colonized by EHEC longer than ruminants fed a grain-based diet (53, 57, 59). Understanding the effect of diet in EHEC colonization of cattle is significant, given that grain diets predominate in feedlots.

An important consideration of the effect of different diets on EHEC cattle colonization has to do with changes in the composition of the GI microbiota (60, 61). Given that EHEC recognizes the AHL chemical signals produced by the rumen microbiota through SdiA to modulate its gene expression toward efficient colonization of cattle (29) and animals fed a hay diet are colonized by EHEC for longer periods of time (Table 3), we wanted to investigate whether SdiA regulation is relevant for EHEC colonization of cattle fed either diets. We previously reported that AHLs are present within the rumen of grain-fed cattle and that such AHLs can decrease expression of the LEE and increase expression of the *gad* genes. Deletion of *sdiA* removes the ability to sense AHLs and regulate LEE and *gad* gene expression, and as a result the *sdiA* mutant did not grow well in rumen and was defective, compared to the WT, for colonization of the RAJ mucosa of these cattle (29). Here, we report that these same animals, after clearing carriage of EHEC and being acclimated to a hay diet, still harbor AHLs in their rumen, and these AHLs can still repress *ler* and increase *gad* gene expression in an SdiA-dependent way (Fig. 2 and 3). The WT EHEC fared better than the *sdiA* mutant in the rumen and the RAJ mucosa of cattle fed either diet (29) (Fig. 4 and

FIG 5 Competition index of the EHEC WT and *sdiA* mutant in hay-fed cattle. Eight 15-month-old Charolais heifers were fed a grain diet or a hay diet and challenged with equal numbers of the parental wild type and the *sdiA* mutant placed directly into the rumen. The ratio of mutant to WT isolates was determined in RAJ. Competitive index (CI) values equal to 1 indicate no difference between strains, values of <1 indicate that the wild type survives/colonizes better than the mutant, and values of >1 indicate that the mutant survives/colonizes better than the wild type. The horizontal line indicates the mean CI value.

5). In the rumen, there were no differences in the competition of the WT and the *sdiA* mutant between the two diets (29) (Fig. 4). However, the WT had a bigger advantage over the *sdiA* mutant for colonization at the RAJ mucosa of animals fed a grain diet compared to those with forage diets (29) (Fig. 5). These data suggest that a grain diet is more conducive to the SdiA-dependent EHEC colonization of cattle in the RAJ.

In these studies, we are not utilizing different populations of heifers fed different diets but rather the same animals acclimated to two different diets (grain and hay), which diminishes variability. We can suggest from our studies that cattle fed a hay diet are colonized by EHEC for a longer period of time and that the SdiA system, although important for EHEC colonization of cattle fed either diet, had a more pronounced role with the grain-fed cattle. Typical finishing diets used in feedlots are $\geq 90\%$ grain, suggesting that interference with this quorum sensing signaling system may be a potential strategy to diminish contamination of meat products, as well as cross-contamination of other food products.

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