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Abrogation of *Salmonella* and *E. coli* O157:H7 in Feedlot Cattle Fed a Proprietary *Saccharomyces cerevisiae* Fermentation Prototype

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Abstract

Salmonella and *E. coli* O157:H7 are insidious problems for the beef industry. Asymptomatic fecal shedding of these pathogens contaminates the hide and carcass. Furthermore, *Salmonella* are unique in their ability to infiltrate lymph nodes leading to the post-harvest contamination of ground beef. These contaminations yield the two most important food safety hazards associated with the consumption of beef. Herein, we report the anti-*Salmonella* and anti-*E. coli* O157:H7 effects of a novel *Saccharomyces cerevisiae* fermentation prototype (**PRT;** NaturSafe™) fed to feedlot cattle. Cattle fed PRT were compared to those fed a combination of monensin, tylosin, and a direct-fed microbial- a standard conventional practice in the U.S. beef industry. In this investigator-blinded study, 1,495 feedlot heifers (300-400 kg) were fed PRT (n=747 heifers) or the standard industry diet (**PC**; n=748 heifers) without PRT for 125-146 days prior to slaughter. At the abattoir, fecal swabs were obtained from 400 animals (n=200/group) and subjected to selective culture for enumerating *Salmonella* and *E. coli* O157:H7. Additionally, subiliac lymph nodes were obtained from 400 carcasses for enumeration of *Salmonella* spp. *Salmonella* isolated from the feces and lymph nodes were subjected to a virulence assay and some antibiotic susceptibility and *Salmonella* serovar testing. When compared to cattle that received PC, *Salmonella* fecal shedding, lymph node infiltration, virulence, and antibiotic resistances were significantly decreased in cattle fed PRT. Additionally, PRT-fed cattle had a lower prevalence of certain *Salmonella* serovars (Newport, Typhimurium, and Dublin) and shed fewer *E. coli* O157:H7. The decrease in *Salmonella* virulence was associated with a decreased expression of *hilA*, a genetic regulator of *Salmonella* invasion into eukaryotic cells. This study revealed that a proprietary *Saccharomyces cerevisiae* fermentation prototype inhibits the shedding, lymph node carriage, downstream virulence, and antibiotic resistance of *Salmonella* residing in cattle beyond the standard conventional practice that includes monensin, tylosin, and a direct-fed microbial.

Introduction

Bovine-associated *Salmonella* are capable of causing clinical disease, but these *Salmonella* can also asymptomatically reside in the intestinal tract and lymph nodes of cattle. Both of these reservoirs create food safety hazards, especially the latter situation where lymph nodes serve as a protective conduit for *Salmonella* passage into ground beef [1]. The intestines also serve as a reservoir for *E. coli* O157:H7, which is non-pathogenic in cattle but highly pathogenic in humans [2].

Intestinal *Salmonella* and *E. coli* O157:H7 lead to surface contamination of beef, which can be mitigated by a number of different strategies at the abattoir [3]. Lymph node infiltration, on the other hand, is problematic given the number of lymph nodes unavoidably co-harvested with ground beef and the covertness of the *Salmonella* within the lymph nodes [1]. Concerns with *Salmonella* in the lymph nodes underscore the need for improving *Salmonella* prophylaxis either in the pre-harvest or post-harvest setting. Recent studies have demonstrated that soluble components present in *Saccharomyces cerevisiae* fermentation products (**SCFP**; SmartCare® and Original XPC™, Diamond V) eliminate *Salmonella* from the gastrointestinal tract of experimentally-infected calves [4]. Specifically, SCFP (SmartCare and Original XPC) significantly reduced the intestinal burden and pathogenic effects of *Salmonella* in calves [4]. Because of these benefits, the anti-*Salmonella* effects of SCFP were examined in feedlot cattle naturally infected with *Salmonella*. The aims of this study were to determine a broad range of effects of a proprietary *S. cerevisiae* fermentation prototype (**PRT;** NaturSafe™) on *Salmonella* when fed to finishing cattle. Specifically fecal shedding, lymph node infiltration, downstream virulence, antibiotic resistance, and the prevalence of virulent *Salmonella* serotypes with a broad host

range were determined. The virulence aspect was examined, in part, because SCFP (EpiCor®, Embria Health Sciences) can increase intestinal butyrate production [5], and this short-chain fatty acid has been shown to inhibit *Salmonella* virulence mechanisms *in vitro* [6]. The antibiotic resistances were examined since SCFP (Original XPC) can modulate the microbiome [7,8], which can alter the antibiograms of resident bacteria [9]. Additionally, we examined the effects of the SCFP on the shedding of *E. coli* O157:H7 since this microbe shares mammalian cell adhesion strategies with *Salmonella* [10]. Comparisons were made to cattle fed a combination of monensin, tylosin, and a direct-fed microbial, which is a standard conventional practice in the U.S. beef industry.

Materials and Methods

Animal care and study design

Heifers (n = 1,495; 300-400kg) were obtained from two sale barns (n=438) and one backgrounding facility (n=1,057) in Kansas in May of 2015. Cattle were shipped to a commercial feedlot in Central Kansas and were provided water and hay *ad libitum*. On day 1 post-arrival,

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heifers were individually weighed, identified, implanted, and vaccinated using standard procedures at the feedlot. Heifers were then randomly assigned into pens in groups of five until each pen reached its optimal capacity (~75 animals) based on bunk space and the area of the pen (14.4 inches of bunk space and 231 square feet of pen space per animal).

Two adjacent pens were designated as a single block and 10 blocks were created within the feedlot. Pens of heifers in each block were provided either a diet that contained a combination of standard industry technologies (PC), including monensin (Rumensin, Elanco Animal Health, 300 mg/head/day), tylosin (Tylovet, Huvepharma, 90 mg/head/day) and a direct-fed microbial (Bovamine Defend, Nutrition Physiology Company, 50 mg/head/day); or a diet containing 18 gm/head/day of a *S. cerevisiae* fermentation prototype (PRT; NaturSafe™, Diamond V) without monensin, tylosin, or the direct-fed microbial. Heifers received three step-up diets prior to their final finishing diet (Table 1). All treatment feed additives were stored under manufacturer-recommend conditions and added to the final ration using a micro-ingredient weight machine (Micro Beef Technologies, Amarillo, TX).

During the study, heifers were monitored for illness and treated as per recommendations by a veterinarian. Heifers that responded to treatment were returned to the study while non-responders were removed from the study. Morbidities and mortalities were indistinct between the two groups (data not shown).

At the conclusion of the study, heifers were shipped 145 miles to a commercial abattoir on two separate dates that were three weeks apart. These shipping dates corresponded to 125 and 146 days on study for the first and second groups, respectively. An equal number of pens per treatment group were shipped on each date (n = 5 per treatment). Shipments and carcass processing occurred on a pen-by-pen basis.

Sample collection at the abattoir

Individual animal numbers were not carried through the entire harvest process. Only the first and last animal within a pen was tagged with a lot identification tag that corresponded back to the pen number. Since individual identification was not maintained, it was not possible to keep track of which animals were sampled. As a result, lymph node and fecal samples may or may not have been collected from the same animals.

Fecal swabs were collected on the rail from 20 animals per pen (replicate). Samples were collected from every third or fourth animal

Ingredient, % of dry matter	Starter	Ration 2	Ration 3	Finisher
Steam-flaked corn	30.2	45.8	58.6	66.1
Wet distiller's grain	22.2	19.5	18.0	18.0
Alfalfa hay	38.0	25.0	13.0	-
Corn stalks	-	-	-	4.0
Corn silage	7.0	7.0	5.0	4.0
Tallow	-	-	1.5	2.9
Supplement[1,2]	2.6	2.7	3.9	5.0

[1] Control rations were formulated to provide 300 mg of monensin (Elanco Animal Health, Greenfield, IN), 90 mg of tylosin (Zoetis Animal Health, Florham, NJ), 0.5 mg of melengestrol acetate (Zoetis Animal Health), and 50 gm Bovamine Defend (Nutrition Physiology Company, Overland Park, KS) per heifer daily throughout the study, and 250 mg of ractopamine hydrochloride (Zoetis Animal Health) per heifer daily during the last 28 days on feed.

[2] Rations containing PRT were formulated to provide 18 gm of a *Saccharomyces cerevisiae* fermentation prototype ((NaturSafe™; Diamond V, Cedar Rapids, IA) and 0.5 mg of melengestrol acetate (Zoetis Animal Health) per heifer daily throughout the study, and 250 mg of ractopamine hydrochloride (Zoetis Animal Health) per heifer daily during the last 28 days on feed.

Table 1: Composition of diets.

within a replicate. Fecal samples were collected using a 3M-sponge stick pre-saturated with buffered peptone water. Sponge sticks were inserted into the rectum (recto-anal junction) to collect the sample. After the sample was collected, the sponge was placed into a pre-labeled bag containing buffered peptone water. The bag was closed and placed into a cooler.

Subiliac lymph nodes and the surrounding tissue were collected post-evisceration. Sample collection began with the first carcass in each replicate and continued with every third or fourth carcass within that replicate. Lymph nodes were placed into pre-labeled Whirlpak bags. The bags were closed and placed in a cooler. Fecal swabs and lymph node samples were then immediately shipped on ice to Iowa State University College of Veterinary Medicine (Ames, Iowa, USA) for microbiological analyses.

Assessment of *Salmonella* fecal load and lymph node infiltration

Salmonella spp. were enumerated from every fecal swab sample and lymph node collected (20 per pen; 200 per treatment) using selective agar (XLD) methods described by Brewer *et al.* [4]. Approximately 0.3 gm of feces or lymph node were collected on a sterile cotton swab and then aseptically transferred into 10mL Lennox broth and an aliquot of the broth was immediately plated on XLD agar, incubated overnight at 37°C, and subjected to enumeration by manual counting of black-centered colonies the next day. Load was then determined as (colonies recovered) × (the dilution factor) / gm of feces or lymph node. Prevalence was calculated as percent of heifers harboring any *Salmonella* and was compiled across pens within a treatment group.

Assessment of *E. coli* O157:H7 in the Feces

E. coli O157:H7 was enumerated in 100 of the swab samples (five per pen; 50 swab samples per treatment) using selective media (Sorbitol-MacConkey agar) and a PCR targeting *E. coli* O157:H7 virulence genes as per Sharma and Casey [11]. Approximately 0.3 gm of feces were transferred into enrichment broth [11] and an aliquot of the broth was plated on sorbitol-MacConkey agar, incubated overnight at 37°C, and subjected to enumeration by manual counting of non-fermenting colonies the next day. From each pen-specific set of agar plates, 96 colonies were selected and subjected to the PCR targeting *E. coli* O157:H7 virulence genes. Load was then determined as (colonies recovered × the dilution factor × the percent of colonies yielding an *E. coli* O157H7-specific amplicon within the pen) / gm of feces. Prevalence was calculated as percent of heifers harboring any *E. coli* O157:H7 within a pen, and was compiled across pens within a treatment group.

Assessment of tissue culture invasiveness by *Salmonella* recovered from cattle

Approximately 50% of the recovered *Salmonella* were subjected to a standard antibiotic protection-based tissue culture invasion assay [12] adapted for use with both antibiotic-susceptible and antibiotic-resistant *Salmonella* [13]. Colonies were collected *en masse*, on a pen-by-pen basis, into nutrient broth and then immediately incubated for 1 hr with HEp-2 tissue culture cells at 37°C. Bacteria were recovered from inside tissue culture cells via cell lysis, incubated on XLD agar overnight at 37°C, and enumerated the next day. Percent invasion was determined as (number of black-centered colonies recovered from inside cells / number of colonies added to cells) × 100.

Assessment of virulence gene expression in *Salmonella* isolated from cattle

In order to correlate the virulence of *Salmonella* recovered from cattle with gene expression events in the pathogen, approximately

10% of the recovered *Salmonella* isolates were subjected to an assay that quantitates the expression of *hilA* (a key regulator of *Salmonella* invasion genes; [14]). RNA was extracted from the isolates that were collected *en masse* on a pen-by-pen basis, and then subjected to a semi-quantitative RT-PCR targeting the *hilA* transcript [15].

Assessment of antibiotic resistance in *Salmonella* recovered from cattle

Approximately 20% of the recovered *Salmonella* were individually subjected to micro-broth assays with individual antibiotics (florfenicol, ceftiofur, and enrofloxacin) at breakpoint concentrations [16]. Colonies that grew in the breakpoint concentrations were deemed to be resistant. Percent resistant were then determined as (number of resistant colonies / number of colonies examined) x 100. Data were compared across pens and between groups.

Assessment of the presence of three important *Salmonella* serotypes in the samples

Nearly 20% of the recovered *Salmonella* were individually subjected to PCR assays that detect the presence of genes related to Dublin [17], Typhimurium [17], and Newport [18] serotypes. Colonies yielding a specific PCR amplicon(s) were deemed to belong to the ascribed serotype. Percent belonging to the serotype were then determined as (number of colonies yielding a specific amplicon / number of colonies examined) × 100. Data were compared across pens and between groups.

Statistical analyses

Statistical comparisons were made using an analysis of variance with Tukey's *ad hoc* test for multiple comparisons (GraphPad Prism, Version 6, La Jolla, CA). Significant differences were defined at $P \leq 0.05$.

Results

### Assessment of *Salmonella* Fecal Shedding in Cattle

Salmonella can be a potential pathogen source for humans who consume improperly cooked beef. Carcasses can become exposed to *Salmonella* through fecal contamination during processing. In this study, fecal shedding of *Salmonella* was evaluated in 200 heifer's postmortem from each treatment group. As shown in Figure 1a, fecal shedding of *Salmonella* was significantly less ($P<0.05$) in cattle fed PRT (105 versus 405 CFU/gm of feces, respectively). The relative prevalence of fecal shedding was significantly less ($P<0.05$) in heifers fed PRT (6 versus 13%, respectively) as per Figure 1b.
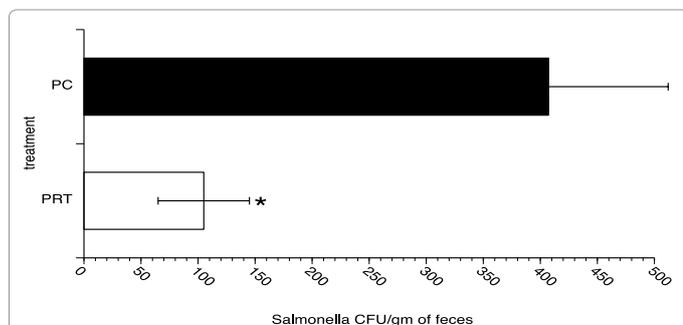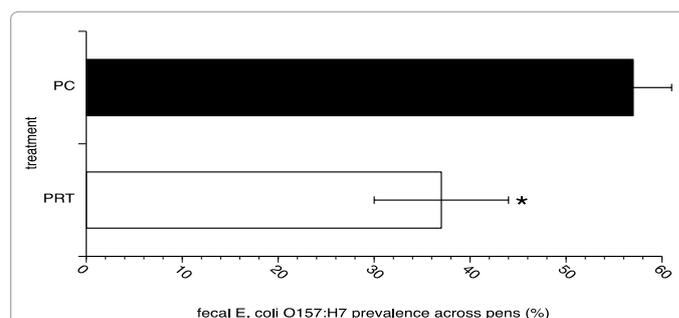

Figure 1a: *Salmonella* fecal load in heifers fed PRT (NaturSafe™) or PC. The PC diet contained monensin (Elanco Animal Health), tylosin (Huvepharma) and a direct-fed microbial (Nutrition Physiology Company). The PRT diet contained *Saccharomyces cerevisiae* fermentation prototype ((NaturSafe™; Diamond V) and no monensin, tylosin, or a direct-fed microbial. Data represent the mean $\pm$ SEM for measurements on 200 heifers in each group. *P<0.05 versus Control.
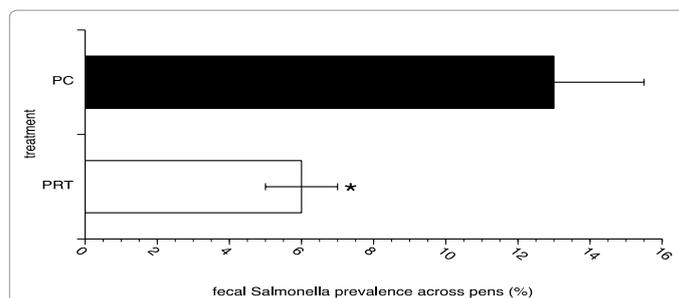
Figure 1b: Prevalence of *Salmonella* fecal shedding in heifers fed PRT (NaturSafe™) or PC. The PC diet contained monensin (Elanco Animal Health), tylosin (Huvepharma) and a direct-fed microbial (Nutrition Physiology Company). The PRT diet contained *Saccharomyces cerevisiae* fermentation prototype (NaturSafe™; Diamond V) and no monensin, tylosin, or a direct-fed microbial. Data represent the mean $\pm$ SEM for the qualitative prevalence (% of heifers shedding *Salmonella*) across pens in each group. *P < 0.05 versus Control.
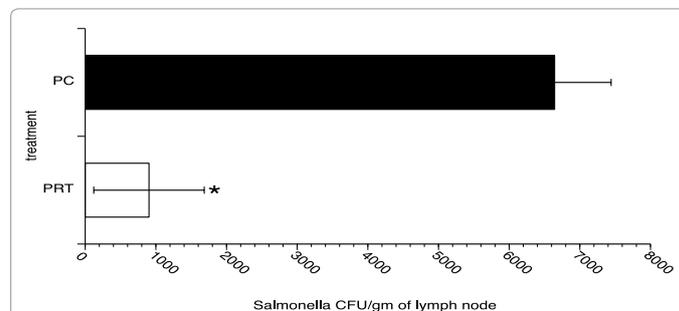
Figure 2a: Assessment of lymph node infiltration by *Salmonella* in heifers fed PRT (NaturSafe™) or PC. The PC diet contained monensin (Elanco Animal Health), tylosin (Huvepharma) and a direct-fed microbial (Nutrition Physiology Company). The PRT diet contained *Saccharomyces cerevisiae* fermentation prototype (NaturSafe™; Diamond V) and no monensin, tylosin, or a direct-fed microbial. Data represent the mean $\pm$ SEM for measurements on 200 heifers in each group. *P<0.05 versus Control.

Figure 2b. Assessment of the prevalence of *Salmonella* lymph node infiltration in heifers fed PRT (NaturSafe™) or PC. The PC diet contained monensin (Elanco Animal Health), tylosin (Huvepharma) and a direct-fed microbial (Nutrition Physiology Company). The PRT diet contained *Saccharomyces cerevisiae* fermentation prototype (NaturSafe™; Diamond V) and no monensin, tylosin, or a direct-fed microbial. Data represent the mean $\pm$ SEM for the qualitative prevalence (% of heifers shedding *Salmonella*) across pens in each group. *P < 0.05 versus Control.

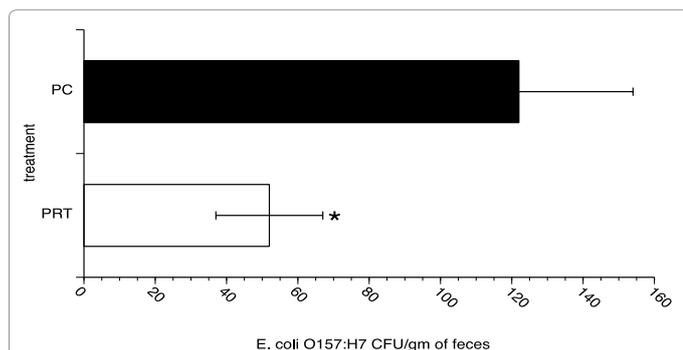### Assessment of lymph node infiltration by *Salmonella*

Since *Salmonella* lymph node carriage can be a source of contamination of ground beef [19], *Salmonella* load was determined in the subiliac lymph nodes of 200 carcasses from each treatment group. As shown in Figure 2a, lymph node infiltration was significantly less ($P<0.05$) in carcasses from heifers fed PRT (902 versus 6,642 CFU/gm of lymph node, respectively). The percent of *Salmonella*-bearing lymph nodes was significantly less ($P<0.05$) in carcasses from heifers fed PRT (4 versus 14%, respectively) as per Figure 2b.

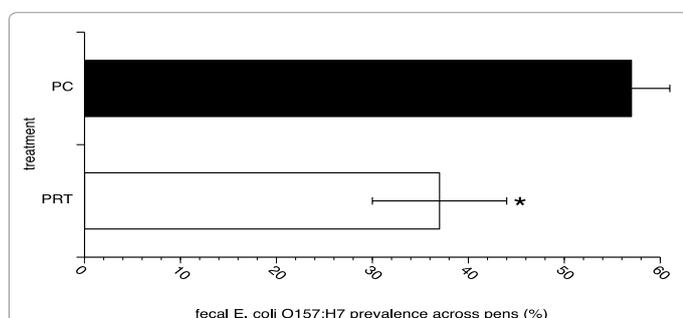## Determination of *E. coli* O157:H7 in the feces of cattle

To determine if PRT had an effect on the presence of *E. coli* O157:H7 in the feces of the heifers, fecal samples (100 per treatment group) were quantitatively examined for the presence of this critical foodborne pathogen. As shown in Figure 3a, heifers fed PRT had a statistically lower ($P<0.05$) *E. coli* O157:H7 fecal load than heifers fed PC (52 versus 122 CFU/gm of feces, respectively). Figure 3b reveals a decreased prevalence ($P<0.05$) of *E. coli* O157:H7 in heifers fed PRT when compared to those receiving the PC diet (37 versus 57%, respectively).

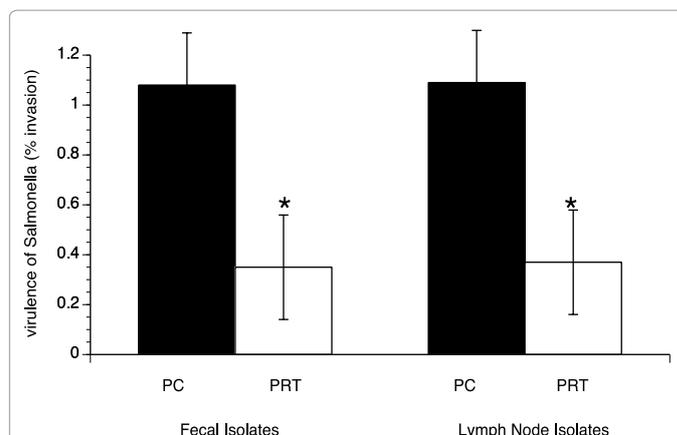## Assessment of the virulence of *Salmonella* recovered from cattle

In order to compare the virulence of *Salmonella* recovered from cattle, the isolates were subjected to an assay that predicts the ability of *Salmonella* to invade gut epithelial cells, which is a hallmark of *Salmonella* virulence [20]. The effects of PRT on virulence were examined due to the ability of SCFP to increase butyrate in the intestine [5]. Research has shown that butyrate can decrease the *Salmonella* invasion gene (*hilA*) expression *in vitro* [6], which results in the decreased ability of *Salmonella* to invade cells. In the current study, invasiveness of *Salmonella* was significantly less ($P<0.05$) in *Salmonella* recovered from the feces and lymph nodes of cattle fed PRT (Figure 4). This decrease in

Figure 4: Tissue culture invasiveness of *Salmonella* recovered from the feces or lymph nodes of heifers fed PRT (NaturSafe™) or PC. The PC diet contained monensin (Elanco Animal Health), tylosin (Huvepharma) and a direct-fed microbial (Nutrition Physiology Company). The PRT diet contained *Saccharomyces cerevisiae* fermentation prototype (NaturSafe™; Diamond V) and no monensin, tylosin, or a direct-fed microbial. Data represent the mean ± SEM for group measurements on *Salmonella* recovered from 200 heifers in each group. *$P<0.05$ versus Control.
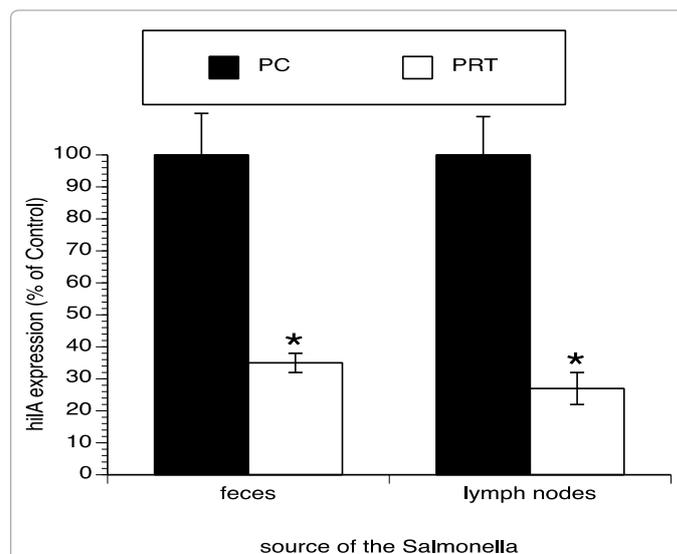

Figure 3a: *E. coli* O157:H7 fecal load in heifers fed PRT (NaturSafe™) or PC. The PC diet contained monensin (Elanco Animal Health), tylosin (Huvepharma) and a direct-fed microbial (Nutrition Physiology Company). The PRT diet contained *Saccharomyces cerevisiae* fermentation prototype (NaturSafe™; Diamond V) and no monensin, tylosin, or a direct-fed microbial. Data represent the mean ± SEM for measurements on 50 heifers in each group. *$P<0.05$ versus Control.

Figure 5: Semi-quantitation of *hilA* expression of *Salmonella* recovered from the feces and lymph nodes of heifers fed PRT (NaturSafe™) or PC. The PC diet contained monensin (Elanco Animal Health), tylosin (Huvepharma) and a direct-fed microbial (Nutrition Physiology Company). The PRT diet contained *Saccharomyces cerevisiae* fermentation prototype (NaturSafe™; Diamond V) and no monensin, tylosin, or a direct-fed microbial. Data represent the mean ± SEM for pooled group measurements on *Salmonella* recovered from feces or lymph nodes. *$P<0.05$ versus Control.

Figure 3b: Prevalence of *E. coli* O157:H7 fecal shedding in heifers fed PRT (NaturSafe™) or PC. The PC diet contained monensin (Elanco Animal Health), tylosin (Huvepharma) and a direct-fed microbial (Nutrition Physiology Company). The PRT diet contained *Saccharomyces cerevisiae* fermentation prototype (NaturSafe™; Diamond V) and no monensin, tylosin, or a direct-fed microbial. Data represent the mean ± SEM for the qualitative prevalence (% of heifers shedding *E. coli* O157:H7) across pens in each group. *$P<0.05$ versus Control.

invasiveness coincided with a decrease in the expression of *hilA* (Figure 5), a major regulator of *Salmonella* virulence for mammalian hosts [14].

Assessment of the antibiotic resistance of *Salmonella* recovered from cattle

The antibiotic resistance of *Salmonella* in lymph nodes and fecal samples were examined since SCFP can modulate the microbiome [7,8], which can alter the antibiograms of resident bacteria [9]. To assess the possibility that PRT inhibits antibiotic resistant *Salmonella* or induces the expulsion of antibiotic resistance elements from *Salmonella*, isolates

recovered from cattle were subjected to an antibiotic susceptibility assay that utilized three individual antibiotics (ceftiofur, enrofloxacin, and florfenicol). These three antibiotics were chosen given their extended spectra and importance in bovine therapeutics. Additionally, two of the three antibiotics tested (ceftiofur and enrofloxacin) have counterparts important for human therapeutics (ceftriaxone and ciprofloxacin, respectively).

Figure 6 reveals a decrease in the prevalence of resistant (*P*<0.05) *Salmonella* recovered from heifers fed PRT for all three antibiotics. This figure represents isolates from both feces and lymph nodes. It is of note that resistance of these antibiotics was, in general, more prevalent in the fecal isolates when compared to the lymph node isolates.

Assessment of the presence of three important *Salmonella* serotypes in the samples

Salmonella isolates recovered from the feces or lymph nodes of cattle were subjected to PCR assays targeting three serotypes (Dublin, Newport, and Typhimurium). As shown in Figure 7, the prevalence of two of these serovars was diminished in heifers fed PRT, regardless of the source of the

Figure 6: Prevalence of antibiotic resistant *Salmonella* recovered from the feces and lymph nodes of heifers fed PRT (NaturSafe™) or PC. The PC diet contained monensin (Elanco Animal Health), tylosin (Huvepharma) and a direct-fed microbial (Nutrition Physiology Company). The PRT diet contained *Saccharomyces cerevisiae* fermentation prototype (NaturSafe™; Diamond V) and no monensin, tylosin, or a direct-fed microbial. Data represent the mean ± SEM for pooled group measurements on *Salmonella* recovered from feces and lymph nodes. *P<0.05 versus Control.

Figure 7: Prevalence of certain *Salmonella* serotypes recovered from the feces and lymph nodes of heifers fed PRT (NaturSafe™) or PC. The PC diet contained monensin (Elanco Animal Health), tylosin (Huvepharma) and a direct-fed microbial (Nutrition Physiology Company). The PRT diet contained *Saccharomyces cerevisiae* fermentation prototype (NaturSafe™; Diamond V) and no monensin, tylosin, or a direct-fed microbial. Data represent the mean ± SEM for group measurements on *Salmonella* recovered from feces and lymph nodes. *P<0.05 versus Control.

isolates. No *S.* Dublin were isolated from feces and only one colony of *S.* Dublin was isolated from lymph nodes. Thus, statistical evaluations are not presented for this minor subsection of the study.

Discussion

Salmonella and *E. coli* O157:H7 are insidious problems for the beef industry and represent critical food safety hazards. *Salmonella* and *E. coli* O157:H7 can be shed in fecal material that can contaminate the carcass during processing. *Salmonella* is also harbored in the lymph nodes, which can lead to contamination of ground beef. Therefore, identifying mitigation strategies for both pathogens is needed especially considering the covert nature of *Salmonella* lymph node infiltration [19].

In this study, the anti-*Salmonella* and anti-*E. coli* O157:H7 effects of NaturSafe™ (PRT) were examined and two critical indicators of *Salmonella* contamination (fecal shedding and lymph node infiltration) were significantly reduced by NaturSafe™. In this study, heifers fed PC shed a higher number of *Salmonella* and *E. coli* O157:H7 and had more *Salmonella* present in the lymph nodes, which ultimately increases the risk of pathogen transmission to humans that ingest beef. There are currently no other research studies looking at the effects of NaturSafe™ on pathogens in beef cattle but there are studies examining the effects of other SCFP products on pathogen load. These results described herein are consistent with a previous study in which SCFP (SmartCare and Original XPC) reduced intestinal colonization in pre-ruminant calves [4], which is possibly due to macrophage-associated clearance of the pathogen. In another study, cannulated beef heifers supplemented with SCFP (Original XP*, Diamond V) had less *E. coli* O157:H7 colonizing the recto-anal junction after being challenged with *E. coli* O157:H7 [21]. Therefore, SCFP products seem to minimize the levels of *Salmonella* and *E. coli* O157:H7 in cattle.

Other significant and unique findings in this study were the reduction in virulence and antibiotic resistance in *Salmonella* recovered from heifers fed PRT. Reduced virulence was detected by diminished tissue culture invasion with a concomitant reduction in the expression of *hilA* [14]. It is unclear how virulence was mitigated by feeding this SCFP prototype. However, previous studies revealed that SCFP (EpiCor) increases the gastrointestinal production of butyrate [5], a putative repressor of *hilA* expression [22]. Regardless of the mechanism underlying this effect, the observed magnitude of decreased invasiveness is likely to increase the infectious dose of *Salmonella* for a human as evidenced by our prior study, in which this level of diminished invasiveness altered the murine LD_{50} approximately 5-fold [13].
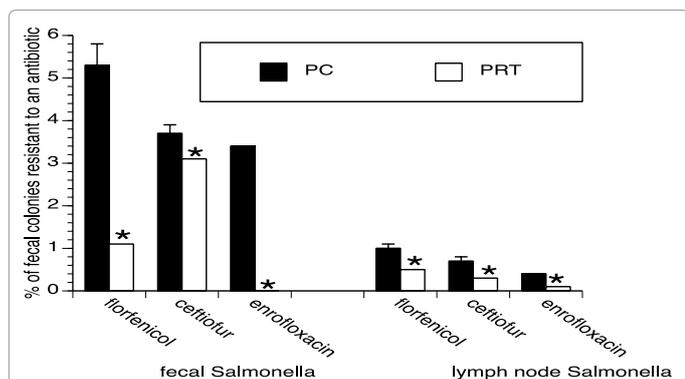
Conclusions

In summary, NaturSafe™-fed feedlot heifers were significantly less likely to shed *Salmonella* and harbor this pathogen in the lymph nodes. The anti-shedding effect of NaturSafe™ was also observed for *E. coli* O157:H7. Additionally, NaturSafe™ reduced the virulence and antibiotic resistance of recovered *Salmonella*. Ultimately, these beneficial effects will have a marked positive effect on food safety in the beef industry. Future mechanistic studies are warranted to uncover the molecular bases for these effects.
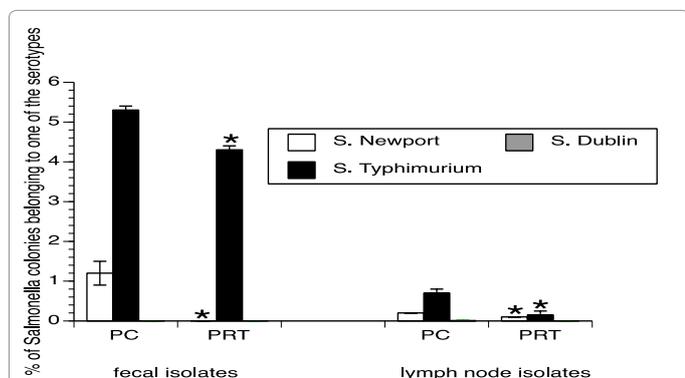
References

1. Brichta-Harhay DM, Arthur TM, Bosilevac JM, Kalchayanand N, Schmidt JW, et al. (2012) Microbiological analysis of bovine lymph nodes for the detection of *Salmonella enterica*. J Food Prot 75: 854-858.

2. Smith JL, Fratamico PM, Gunther NW 4th (2014) Shiga toxin-producing *Escherichia coli*. Adv Appl Microbiol 86: 145-197.

3. Soon JM, Chadd SA, Baines RN (2011) *Escherichia coli* O157:H7 in beef cattle: on farm contamination and pre-slaughter control methods. Anim Health Res Rev 12: 197-211.

4. Brewer MT, Anderson KL, Yoon I, Scott MF, Carlson SA (2014) Amelioration of salmonellosis in pre-weaned dairy calves fed *Saccharomyces cerevisiae* fermentation products in feed and milk replacer. Vet Microbiol 172: 248-255.

5. Possemiers S, Pinheiro I, Verhelst A, Van den Abbeele P, Maignien L, et al. (2013) A dried yeast fermentate selectively modulates both the luminal and mucosal gut microbiota and protects against inflammation, as studied in an integrated *in vitro* approach. J Agric Food Chem 61: 9380-9392.

6. Gantois I, Ducatelle R, Pasmans F, Haesebrouck F, Hautefort I, et al. (2006) Butyrate specifically down-regulates *Salmonella* pathogenicity island 1 gene expression. Appl Environ Microbiol 72: 946-949.

7. Mullins CR, Mamedova LK, Carpenter AJ, Ying Y, Allen MS, et al. (2013) Analysis of rumen microbial populations in lactating dairy cattle fed diets varying in carbohydrate profiles and *Saccharomyces cerevisiae* fermentation product. J Dairy Sci 96: 5872-5881.

8. Price KL, Totty HR, Lee HB, Utt MD, Fitzner GE, et al. (2010) Use of *Saccharomyces cerevisiae* fermentation product on growth performance and microbiota of weaned pigs during *Salmonella* infection. J Anim Sci 88: 3896-3908.

9. Kirkup BC, Riley MA (2004) Antibiotic-mediated antagonism leads to a bacterial game of rock-paper-scissors *in vivo*. Nature 428: 412-414.

10. Rossez Y, Wolfson EB, Holmes A, Gally DL, Holden NJ (2015) Bacterial flagella: twist and stick, or dodge across the kingdoms. PLoS Pathog 11: e1004483.

11. Sharma VK, Casey TA (2014) *Escherichia coli* O157:H7 lacking the qseBC-encoded quorum-sensing system outcompetes the parental strain in colonization of cattle intestines. Appl Environ Microbiol 80: 1882-1892.

12. Giannella RA, Washington O, Gemski P, Formal SB (1973) Invasion of HeLa cells by *Salmonella typhimurium*: a model for study of invasiveness of *Salmonella*. J Infect Dis 128: 69-75.

13. Carlson SA, Browning M, Ferris KE, Jones BD (2000) Identification of diminished tissue culture invasiveness among multiple antibiotic resistant *Salmonella typhimurium* DT104. Microb Pathog 28: 37-44.

14. Bajaj V, Hwang C, Lee CA (1995) hilA is a novel ompR/toxR family member that activates the expression of *Salmonella typhimurium* invasion genes. Mol Microbiol 18: 715-727.

15. Carlson SA, Sharma VK, McCuddin ZP, Rasmussen MA, Franklin SK (2007) Involvement of a *Salmonella* genomic island 1 gene in the rumen protozoan-mediated enhancement of invasion for multiple-antibiotic-resistant *Salmonella enterica* serovar *Typhimurium*. Infect Immun 75: 792-800.

16. Clinical and Laboratory Standards Institute (2015) Performance standards for antimicrobial disk and dilution susceptibility tests. Volume 29. 2nd edn, Wayne, PA, USA.

17. Akiba M, Kusumoto M, Iwata T (2011) Rapid identification of *Salmonella enterica* serovars, Typhimurium, Choleraesuis, Infantis, Hadar, Enteritidis, Dublin and Gallinarum, by multiplex PCR. J Microbiol Methods 85: 9-15.

18. Cao G, Meng J, Strain E, Stones R, Pettengill J, et al. (2013) Phylogenetics and differentiation of *Salmonella* Newport lineages by whole genome sequencing. PLoS One 8: e55687.

19. Brichta-Harhay DM, Guerini MN, Arthur TM, Bosilevac JM, Kalchayanand N, et al. (2008) *Salmonella* and *Escherichia coli* O157:H7 contamination on hides and carcasses of cull cattle presented for slaughter in the United States: an evaluation of prevalence and bacterial loads by immunomagnetic separation and direct plating methods. Appl Environ Microbiol 74: 6289-6297.

20. Millemann Y (1998) Pathogenic power of *Salmonellae*: virulence factors and study models. Vet Res 29: 385-407.

21. Liou L, Sheng H, Ferens W, Schneider C, Hristov A, et al. (2009) Reduced Carriage of *Escherichia coli* O157:H7 in Cattle Fed Yeast Culture Supplement. The Professional Animal Scientist 25: 553-558.

22. Durant JA, Corrier DE, Ricke SC (2000) Short-chain volatile fatty acids modulate the expression of the hilA and invF genes of *Salmonella typhimurium*. J Food Prot 63: 573-578.