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Matthew T. Brewer  
*Iowa State University*, brewermt@iastate.edu

Kristi L. Anderson  
*Iowa State University*, kristia@iastate.edu

Ilkyu Yoon  
*Diamond V*

Mark F. Scott  
*Diamond V*

Steve A. Carlson  
*Iowa State University*, stevec@iastate.edu

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Amelioration of salmonellosis in pre-weaned dairy calves fed Saccharomyces cerevisiae fermentation products in feed and milk replacer

Matthew T. Brewer a, Kristi L. Anderson a, Ilkyu Yoon b, Mark F. Scott b, Steve A. Carlson a, *

a Department of Biomedical Sciences, Iowa State University College of Veterinary Medicine, Ames, IA 50011, United States
b Diamond V, 2525 60th Avenue SW, Cedar Rapids, IA 52404, United States

ABSTRACT

Salmonellosis is an insidious and potentially epidemic problem in pre-weaned dairy calves. Managing this disease, or any other diarrheal disease, is a financial burden to producers. Calf mortalities and medicinal treatments are overt costs of salmonellosis, while hidden costs include hampered weight gains and persistent intestinal colonization of the pathogen. In this study, we examined the anti-Salmonella effects of Saccharomyces cerevisiae fermentation products (SCFP) incorporated into both the milk replacer and the starter grain. In a blinded study, 2–8 day-old calves were fed SCFP (n = 20 calves) or an SCFP-free Control (n = 20 calves) for two weeks before and three weeks after experimental challenge with Salmonella enterica serotype Typhimurium. Following the challenge, calves were monitored for clinical signs and parameters associated with salmonellosis. Calves were then euthanized and examined for rumen development and intestinal Salmonella colonization. When compared to calves that received milk replacer and feed lacking SCFP, calves fed SCFP had fewer bouts of diarrhea and fever. Rumens from these calves were more developed, as measured by the length of papillae, which is consistent with the enhanced weight gain observed in this treatment group. Additionally, Salmonella intestinal colonization was reduced in SCFP-fed calves and Salmonella fecal shedding disappeared at an earlier stage in these calves. This study revealed that the combination of two proprietary S. cerevisiae fermentation products provide marked benefit for preventing the negative effects of salmonellosis in pre-weaned dairy calves, while also boosting productivity. The mechanism of action needs to be clarified, but it may be related to the observed decrease in colonization by the pathogen and increase in rumen development.

1. Introduction

Salmonellosis is one of the many diarrheal diseases affecting pre-weaned dairy calves. Salmonella organisms are commonly isolated from dairy farms (Fossler et al., 2004) and the fecal-oral transmission route can occur from dam to offspring. Calves can also acquire the organism from fecal-contaminated fomites or the environment. Calves manifest the disease as diarrhea, fever, anorexia, and dehydration all of which significantly compromise the development and maturation of the animal. Further costs include treatment with electrolytes or antibiotics or both, and some calves still perish because of the increasing prevalence of antibiotic resistance in Salmonella (Cummings et al., 2013) and hypervirulence associated with
multi-resistant strains (Rasmussen et al., 2005). Furthermore, calves that survive salmonellosis can be long-term carriers of the pathogen (Nielsen et al., 2012) and these adult animals can serve as a persistent source for new infections in the herd (Cobbold et al., 2006). Environmental persistence also contributes to this problem (Cobbold et al., 2006).

Preventing Salmonella infections currently focuses on a vaccine technology (Hermesch et al., 2008). Unfortunately, this vaccine is only for cattle that are six months or older thus pre-weaned calves are dependent upon colostral passive immunization from the vaccinated dam. Anti-Salmonella bacteria have been tried but are frequently unsuccessful because of the immunodominance of the Salmonella O-antigen (Barat et al., 2012) and serovar specificity (House et al., 2001). Anti-lipopolysaccharide antiserum and lipopolysaccharide toxoids are available but the anti-Salmonella benefits have not been clearly established. Thus Salmonella prophylaxis is not optimal at this time in the pre-weaned calf (Lanzas et al., 2008), although vaccinating the dam will reduce the environmental exposure of the calf.

It has been shown that soluble components present in Saccharomyces cerevisiae fermentation products (SCFP) enhance gut health (Jensen et al., 2008a) and promote immune function (Jensen et al., 2007). When supplemented to the starter grain, SCFP improved rumen development, starter grain intake, and BW gain of non-challenged calves (Lesmeister et al., 2004). Additionally, SCFP was shown to improve the gastrointestinal health of calves in a Salmonella endemic herd (Magalhães et al., 2008). Because of these benefits associated with SCFP, we examined its anti-Salmonella effects when fed to pre-weaned dairy calves experimentally infected with Salmonella. The specific aims of this study were to determine the effects of the combination of two proprietary SCFP (Diamond V SmartCare™ and Diamond V Original XPC™) on the growth and rumen development, clinical signs of salmonellosis, Salmonella shedding, and intestinal colonization of Salmonella in pre-weaned dairy calves experimentally infected with Salmonella.

2. Materials and methods

2.1. Calves and pre-infection treatments

Animal experiments were approved by the Animal Care and Use Committee at Iowa State University. Forty Holstein or Holstein-cross calves (32 females and 8 males) were purchased from a local supplier in northwest Iowa. Calves were fed colostrum for the first two days after birth and then fed a standard milk replacer until shipment to Iowa State University at 2–8 days of age. Upon arrival at an animal biosafety level-2 building at Iowa State University, calves were weighed (28–47 kg, with Holstein–Jersey crosses representing the lower weights) and randomly assigned (without redistribution) to one of two separate but adjacent rooms. Each room had constant ambient temperatures (about 22 °C) and humidity (about 40%) and was ventilated by negative pressure through HEPA filters. Calves were housed in individual 18 m² pens on Tenderfoot-type flooring without bedding.

Two separate experiments were performed each using 20 calves (10 per group) of similar ages (2–4 days in one experiment and 6–8 days in the other experiment), and treatment groups were alternated in the two different rooms in each experiment in order to avoid a “room effect”. Each room was fed either SCFP or the Control to avoid the potential for inappropriate administration of a treatment within a room. Rooms were alternated between the two experiments, i.e., in the first experiment calves in “Room A” received SCFP while calves in this same room received the Control in the second experiment.

Calves were randomly assigned to one of two treatments: Control (no additive in milk replacer or starter grain) or diet that contained two proprietary S. cerevisiae fermentation products delivered separately (SCFP; 1 g/head/d SmartCare™ [0.15% inclusion rate in conventional milk replacer] and 3.5 g/head/d Original XPC™ administered orally via gelatin capsule; Diamond V, Cedar Rapids, Iowa). SmartCare is a water dispersible product that can be added directly to milk or milk replacer as a supplement for pre-weaning liquid calf diets (starting at d 1). Original XPC is dry feed product commonly used in pre-weaning calf starter diets. The combination of these products is the basis for Diamond V’s dairy calf program during the pre-weaning phase. A gelatin capsule containing 3.5 g/head/d grain matrix used to produce XPC was given to Control calves to equalize the nutrients, although minimal, contributed by XPC.

All calves were fed a non-medicated milk replacer (20% all-milk protein, 20% fat; Land O’Lakes Animal Milk Products, Shoreview, MN) at a volume equivalent rate of approximately 10% of arrival BW twice per day (i.e., 5% of BW each feeding) for the duration of the trial. Milk replacer was mixed in single batches using warm water and a cordless drill-driven stirrer. Specifically, each calf received 6 oz of milk replacer in 1 qt water bid, in which the milk replacer was 18.8% (w/v) of the solution.

Calves were fed calf starter (Calf Startena™, 18% crude protein, 0.005% decoquinate, Purina Mills, LLC, St. Louis, MO) and water ad libitum, although it was not feasible to measure intake of either because of spillage and other uncontrollable factors. The Iowa State University investigators (M.T.B., K.L.A., and S.A.C.) were blinded as to which calves received the Control or SCFP treatments. Specifically, the Diamond V investigators (I.Y. and M.F.S.) notified a third party (scientists at the Office of Intellectual Property at Iowa State University) as to the identity of the treatment groups prior to the onset of the studies. Once the studies were completed, the Iowa State University investigators revealed the data to the Office of Intellectual Property who then revealed the identity of the treatment groups.

In the two-week pre-infection phase, six to seven calves from each group were orally treated with one dose of sulfamethazine (356 mg/kg; Sustain™, Bimeda, Oakbrook Terrace, IL) for veterinarian diagnosed coccidial infections manifested by blood in the feces. This treatment alleviated the bloody feces within 3 d of treatment. Treatment with sulfamethazine was deemed to not have a negative effect on the outcome of the trial since the Salmonella strain used
in this study is resistant to sulfonamides and equivalent numbers of calves from each experimental group were subjected to sulfamethazine treatment.

2.2. *Salmonella* infection of calves

Calves were confirmed to be *Salmonella*-free by fecal culture on arrival and on d 7 and 12 post-arrival. Specifically, 1 g of freshly voided feces was incubated in 20 mL of Lennox L broth (Invitrogen, Carlsbad, CA). After settling, an aliquot (100 μL) of this mixture was streaked onto and then incubated overnight at 37 °C on XLD agar (Fisher Scientific, Pittsburgh, PA) selective for *Salmonella* that appear as red colonies with black centers. All pre-infection fecal samples were free of *Salmonella*. Sulfamethazine was not used in the XLD agar for pre-infection assessment. SL1344 was plated on XLD as a positive control during the experiments.

At d 14 post-infection (d 0 post-infection), calves were orally inoculated with *Salmonella enterica* serotype Typhimurium strain SL1344 (Wray and Sojka, 1978; personal collection of S.A.C.) at the dose of 2 × 10⁶ CFU/kg BW (Xiong et al., 2013). The *Salmonella* inoculum was prepared and dosed as described previously by the Carlson laboratory (Xiong et al., 2013). The inoculum was placed in the gelatin capsule containing the Control or SCFP, which was administered using a small balling gun. Our empirical studies revealed that the strain SL1344 was viable after incubation with either the Original XPC™ or the Control treatment. That is, pre-inoculated experiments revealed that SL1344 was 100% viable and recoverable after remaining in the Control- or SCFP-containing gelatin capsule for 24 h. Additionally, the virulence of SL1344 was retained (as determined by a tissue culture invasion assay) following recovery from the Control- or SCFP-containing capsule (data not shown).

2.3. Assessment of clinical parameters in calves

Pyrexia and diarrhea are frequently observed components of salmonellosis in calves (Smith et al., 1979). On d 0, 1, 2, 3, 4, 5, 6, 7, 10, and 21 post-infection, rectal temperatures were measured and diarrhea was assessed on an ordinal scale. Diarrhea scoring was as follows: 0, no diarrhea; 1, mild diarrhea; 2, profuse diarrhea; or 3, profuse diarrhea with blood. This determination was performed by one investigator (S.A.C.), who was blinded to the treatment groups, with vast experience with experimental salmonellosis.

2.4. Fecal shedding of *Salmonella* shedding in calves

Fecal shedding of *Salmonella* is a highly variable and sporadic occurrence in calves infected with *Salmonella* (Kirchner et al., 2012). Nonetheless, this event is of importance for the spread of this pathogen (Lanzas et al., 2010). On d 0, 1, 2, 3, 4, 5, 6, 7, 10, and 21 post-infection, 1 g of freshly voided feces was briefly vortexed in 20 mL of Lennox L broth (Invitrogen, Carlsbad, CA). An aliquot of this mixture (100 μL) was incubated overnight at 37 °C on XLD agar containing the Committee on Laboratory Standards Institute-derived (CLSI, 2008) breakpoint concentration (512 μg/mL) of sulfamethazine (Sigma–Aldrich, St. Louis, MO), i.e., a concentration that will enable the growth of SL1344 but will inhibit the growth of many other enteric bacteria. The following day red colonies with black centers were enumerated and CFU/g of feces was calculated based on a dilution factor equal to 200.

2.5. Assessment of intestinal colonization by *Salmonella*

Intestinal colonization by *Salmonella* contributes to the persistence of the pathogen (Cobbold et al., 2006) and the fecal shedding that transmits the microbe to other cattle. On d 21 post-infection, all calves were euthanized using xylazine (0.5 mg/kg, intramuscular, Lloyd Laboratories, Walnut, CA) and pentobarbital (100 mg/kg, intravenous, Fort Dodge Laboratories, Fort Dodge, IA). A 2 cm section (approximately 1 g) of distal ileum was aseptically removed from each calf and cut longitudinally. Each section was placed in 20 mL Lennox L broth (Invitrogen, Carlsbad, CA) and briefly vortexed to dislodge the *Salmonella*. An aliquot (100 μL) of this mixture was then dispensed onto XLD agar containing sulfamethazine (to prevent the growth of other bacteria) that were incubated overnight at 37 °C. The following day red colonies with black centers were enumerated and CFU/g of ileum was calculated based on a dilution factor equal to 200.

2.6. Assessment of rumen development in calves

*Salmonella*, and any other enteric pathogen, can have a negative impact upon performance in the pre-weaned calf. It was hypothesized that SCFP may abrogate salmonellosis by promoting overall gastrointestinal health and improving rumen development in pre-weaned calves. Therefore, both weight gain and the size of rumen papillae were assessed in *Salmonella*-infected calves fed SCFP. Following euthanasia, a 4 cm² section of a ventral lateral portion of the rumen was removed and placed in 10% buffered-neutral formalin. Rumen tissues were submitted to the Histopathology Laboratory at the College of Veterinary Medicine at Iowa State University. Tissues were prepared using standard hematoxylin and eosin staining. Length and width of rumen papillae were measured using an intra-ocular ruler. Measurements were collected from 10 randomly selected papillae present on two different sections (i.e., five papillae from each section). Widths were measured at mid-shaft.

2.7. Statistical analyses

For data in which assessments were performed on multiple days (rectal temperatures, diarrhea scores, and fecal shedding), statistical comparisons were made using a repeated measures analysis of variance with Tukey’s ad hoc test for multiple comparisons (GraphPad Prism, Version 6, La Jolla, CA). For data involving single measurements from each calf (intestinal colonization and rumen papillae length), statistical comparisons were performed using a Student’s t-test (GraphPad). Significant differences were defined at P ≤ 0.05. Statistical trends
were consistent when the two sets of experiments were examined independently (data not shown).

3. Results

3.1. Assessment of clinical parameters in calves

Rectal temperatures and diarrhea we monitored in the calves on d 0, 1–7, 10, and 21 post-infection. As shown in Figs. 1 and 2, pyrexia (rectal temperature > 39.2 °C) and diarrhea were observed less frequently (P < 0.05) in calves fed SCFP. The largest differences between calves supplemented with SCFP and Control calves were observed on d 3–5 post-infection for rectal temperatures (38.8–39.0 °C versus 39.5–39.7 °C, respectively), and d 3–7 post-infection for diarrhea scores (0.10–0.25 versus 0.61–0.86 arbitrary units, respectively). None of the SCFP-fed calves exhibited pyrexia at anytime throughout the study, while nearly all Control calves exhibited pyrexia on d 2–6 post-infection. The relative incidence of diarrhea was less in SCFP-fed calves on all days except day 0 (Fig. 2b).

3.2. Assessment of Salmonella shedding in the calves

Salmonella fecal shedding was monitored on d 0, 1–7, 10, and 21 post-infection. As shown in Fig. 3a, there was a quantitative difference in fecal shedding between the two groups of calves on d 6 post-infection in which shedding was less (P < 0.05) in calves fed SCFP (undetectable versus 955 CFU/g of feces). There also was a qualitative difference in fecal shedding of Salmonella on d 3–7 post-infection (Fig. 3b).

3.3. Assessment of adherent Salmonella in the intestines of the calves

Upon euthanasia, ileal sections were excised and subjected to Salmonella culture and enumeration that
quantifies intestinal colonization of Salmonella. As shown in Fig. 4, fewer \( (P < 0.05) \) adherent Salmonella were present in the ilea of calves fed SCFP (1620 versus 9289 CFU/g of ileum). Gross examinations of the ilea revealed no apparent differences between the two groups.

3.4. Assessment of rumen development and weight gain in the calves

As shown in Figs. 5 and 6, rumen papillae length was greater \( (P < 0.05) \) in calves fed SCFP (236 versus 203 \( \mu \)m) while papillae width was indistinguishable (50 versus 40 \( \mu \)m). Table 1 shows the body weight at different stages of experiment and Fig. 7 reveals the superior \( (P < 0.05) \) weight gain in Salmonella-infected calves fed SCFP (23.8 versus 17.2%).

4. Discussion

Salmonella is an insidious problem for the dairy industry. This problem represents a critical animal health issue since cattle of all ages are affected by the pathogen. Adult cattle will exhibit diarrhea and anorexia, both of which compromise the performance of the animal. In the pre-weaned calf, Salmonella can cause diarrhea and malaise that will also hamper performance and expose caretakers to the pathogen. Furthermore, salmonellosis in the pre-weaned calf can lead to
persistent infection and the carrier state. These animals become an asymptomatic source of *Salmonella* for the herd, while some animals may perish because of the infection.

Identifying *Salmonella* mitigation strategies is of critical significance yet the progress is very slow. In this study, the anti-*Salmonella* effects of SCFP were examined and three critical indicators of salmonellosis (pyrexia, diarrhea, and intestinal colonization) were significantly reduced by SCFP. The absence of pyrexia and the diminished diarrhea in the calves fed SCFP are consistent with the reduced intestinal colonization by *Salmonella*. Both pyrexia and diarrhea are dependent upon pathogen burden and it appears that *Salmonella* may be less efficient at attaching to the intestinal tract in the presence of SCFP. This diminished intestinal colonization was manifested by a cessation of *Salmonella* shedding in calves fed SCFP at 4 d prior to the last day of fecal shedding in calves fed the Control. In Control-fed calves, a higher number of adherent *Salmonella* were present in the intestinal tract thus extending the overall shedding period, which ultimately increased the risk of disease transmission. While we did not measure intracellular bacteria in the gut epithelium, we do not envision that differences in intracellular bacteria would account for a five-fold difference in the recovery of the *Salmonella* in the two groups.

Our results are consistent with a previous study (Magalhães et al., 2008) in which feeding SCFP led to an improvement of gastrointestinal health in pre-weaned dairy calves naturally exposed to *Salmonella*. Although the mechanism of action is yet to be clarified, the effect of SCFP on intestinal colonization (Ibukic et al., 2012) and growth
Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body Weights (mean ± SEM) (kg)</th>
<th>% Growth (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d 0 (start)</td>
<td>d 14 (at challenge)</td>
</tr>
<tr>
<td>Control</td>
<td>42.8 ± 0.7</td>
<td>45.1 ± 0.7</td>
</tr>
<tr>
<td>SCFP</td>
<td>40.3 ± 1</td>
<td>42.5 ± 1.1</td>
</tr>
</tbody>
</table>

* Salmonella enterica serotype Typhimurium strain SL1344 at the dose of 2 × 10⁶ CFU/kg BW.

b Control — no additive in milk replacer or starter grain; SCFP — diet that contained two proprietary SCFP products (1 g/head/day SmartCare™ [0.15% inclusion rate in conventional milk replacer] and 3.5 g/head/day Original XPC™ administered orally via gelatin capsule; Diamond V, Cedar Rapids, Iowa).

* P < 0.05 versus Control when comparing growth rates.

(Broomhead et al., 2012; Nserekou et al., 2013) of Salmonella has also been reported in poultry.

Other significant findings in this study are the improved weight gain and rumen papillae maturation in calves fed SCFP, which is consistent with a non-infectious study whereby supplemental SCFP improved pre-weaning calf growth, feed intake, and corresponding rumen development parameters (Lesmeister et al., 2004). Although it is unclear how these parameters were improved by feeding the S. cerevisiae fermentation products, these findings suggest an economic benefit for inclusion in milk replacer and starter grain as demonstrated previously (Magalhães et al., 2008). It is likely that these benefits extend to calves in herds even in which Salmonella is not endemic. Furthermore, it is possible that these benefits extend to protection from related enteric pathogens such as Escherichia coli since previously reported studies suggested SCFP could inhibit the growth of E. coli (Jensen et al., 2008b). Future studies will assess this possibility.

5. Conclusions

In summary, Salmonella-infected dairy calves were significantly less likely to exhibit clinical signs associated with salmonellosis when fed SCFP. Specifically, these calves were less likely to exhibit pyrexia and diarrhea, possibly as a direct result of diminished intestinal colonization by Salmonella. Ultimately, these protective effects augmented growth and improved rumen development in the calves infected with a serious enteric pathogen.

Conflict of interest

This work was funded by Diamond V, along with partial matching funds from the Institute of Physical Research and Technology at Iowa State University. Salary support was provided to the corresponding author in exchange for his time devoted to the project, but no other financial compensation was or will be awarded. Thus the authors declare no conflict of interest.

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