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

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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

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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* bacterins 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 m^2 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