Effects of an Oral Rehydration Solution with Added Bovine Serum Proteins on Small Intestinal Absorptive Capacity

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Summary and Implications

Young calves commonly become infected with viruses and bacteria that damage the intestinal lining. To enhance recovery of small intestinal function following a coronavirus challenge, bovine serum proteins, containing IgG, TGF-β and other growth factors, were added to an oral rehydration solution (ORS) for 32 Holstein and Jersey calves. Calves were housed individually and offered water ad libitum and milk replacer at 10% of BW daily. Control calves were fed a control ORS (CON). The treated calves were fed an ORS that consisted of CON with added bovine serum proteins (GFR). After a 2 d adjustment, calves were orally challenged with a moderately virulent bovine coronavirus isolate. Xylose (0.5 g/kg of BW) was administered orally once daily for 6 d to assess small intestinal recovery. Hematocrits, fecal dry matter, rectal temperatures, attitude scores and dehydration scores were recorded once daily. Concentrations of serum xylose increased with time post-dosing, but did not vary by treatment. Hematocrits and other clinical scores were not significantly different (P >0.05) between treatments. In this model, bovine serum proteins did not appear to enhance intestinal recovery from a coronavirus challenge when added to ORS.

Introduction

Neonatal calf diarrhea is a major cause of economic loss in the dairy industry. Coronavirus is one of the more common pathogens causing calf scours. Pathogens that affect mucosal function of the intestinal tissues, such as coronavirus, destroy villus architecture and decrease absorptive surface area of the small intestine. Loss of epithelial function results in malabsorption of water, sodium, and chloride. Continued diarrhea increases the risk of dehydration and hypoglycemia and, if not treated, eventually leads to death.

When calves develop diarrhea, treatment with oral or intravenous fluid therapy may aid in avoiding problems such as metabolic acidosis. In addition, oral rehydration solutions (ORS) are formulated to maintain hydration, replace lost electrolytes, and provide an energy source for the diarrheic calf. Small intestinal epithelium that has been damaged due to pathogens is one of the most sensitive organs to additions of growth factors. Calves challenged with *Cryptosporidium parvum* have reduced gut permeability when dosed orally with bovine serum concentrate containing elevated amounts of immunoglobulin G (IgG), insulin-like growth factor-I (IGF-I), transforming growth factor-, 1 (TGF-,1) and transforming growth factor-, 2 (TGF-, 2). Colonic cells treated with TGF-, 1 before challenge with *Cryptosporidium parvum* maintain their mucosal barrier as well as membrane integrity.

Therefore, the objective of this study was to enhance the rate of recovery of small intestinal absorptive function following a coronavirus challenge by supplementing an oral rehydration solution with a bovine serum fraction containing elevated levels of growth factors.

Procedures

The trial utilized 32 Jersey and Holstein male calves (3-5 d of age) purchased from sale barns. Following arrival, calves were randomized into 8 blocks of 4 calves and weighed before being placed in individual plastic hutches bedded with wood shavings. Four pints of commercial ORS were fed on arrival. Calves were offered water ad libitum and fed a complete (20% fat and 20% protein) commercial milk replacer at a rate of 5% of bodyweight, twice daily. Feed refusals were monitored and recorded. No hay or starter was offered.

Treatments began approximately 12 h after viral challenge, and were provided at 10% of initial bodyweight, twice daily, for 3 d. Treatment one (CON) calves were fed an oral rehydration solution formulated to include 4g/lb of ascorbic acid (Table 1). Treatment two (GFR) calves were fed CON with the addition of a serum protein fraction consisting of elevated levels of IgG, IGF-I, TGF-, 1 and TGF-, 2 (Table 2). Batches were mixed daily by treatment order to minimize risk of contamination.

Fecal samples were obtained and analyzed daily for dry matter content. Starting on the second day, xylose was fed orally at 0.5 g/kg of BW. Jugular blood samples were obtained prior to xylose feeding to provide baseline values at time 0 and then after xylose feeding at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, and 4 h. An additional 3 cc sample of venous blood was obtained into a heparinized tube at time 0 during collection days and immediately analyzed for hematocrit. Serum was separated from the sample by centrifugation at 3600 *x* g for 15 minutes, collected, and frozen at -20 °F for later analysis of xylose and haptoglobin concentrations. Xylose concentrations were determined spectrophotometrically. Haptoglobin was determined spectrophotometrically using a commercial kit (TriDelta LTD., Morris Plains). Xylose was dosed and serial blood samples collected on d 1, 2, 3, 4, 5, and 6. Calves were challenged orally with bovine coronavirus (5×10^6 plaque forming units of a moderately virulent coronavirus isolate) following the 4 h blood collection period on d 2. Treatments were provided on d 3, 4, and 5.

All performance scores were evaluated and recorded daily by a single person; these scores included fecal scores on a four-point scale (1=firm, 2=soft, 3=runny, 4=watery), attitude scores on a four-point scale (1=alert, 2=slightly depressed, 3=depressed, 4=laterally recumbent), dehydration scores

using skin tent time based on a three-point scale (1=<1s, 2=2-3s, 3=>3s), and a score based on rectal temperature (1=100-102.5, 2=<99.9, 3=>102.6).

Analysis of variance was performed using the mixed procedures of SAS 8.1 and a model for a completely randomized design. The xylose data were log transformed and sorted by treatment. Mean values of data from days 1 and 2 prior to viral challenge were used as a covariate in the model. Least squares means were used to evaluate treatment differences for xylose concentrations, performance parameters and haptoglobin values. Significance was declared at $P < 0.05$ and trends at $P < 0.15$. Treatment means were compared using the method of least significant differences.

Results and Discussion

Changes in xylose concentrations (mg/dl) for calves grouped by day are shown in Figure 1. Treatment values for xylose concentrations in all figures represent covariate-adjusted least squares means. There were no significant differences ($P > 0.05$) in xylose concentrations between treatment groups for any day post-viral challenge.

Least squares means of fecal scores were compared. There were no significant differences between treatment groups, although all treatment groups increased numerically on day 2, before viral challenge. There were no significant differences between least squares means of fecal dry matter between treatment groups, although dry matter was numerically lower for all treatment groups on day 2, correlating to the observed increase in scour scores. There were no significant differences in dehydration scores, attitude scores, hematocrit or temperature between treatments, and no significant differences in haptoglobin concentrations between treatments for any time periods.

In the present study, there were no significant differences in xylose uptake from the small intestine between treatment groups. Mean peak xylose concentrations in the current study ranged from 23 to 40 mg/dl throughout all sampling times and numerically increased following treatments post-challenge, indicating some improvement in gut function. In other studies, xylose concentrations in calves with intestinal damage peaks at 45 to 75 mg/dl. In healthy dogs, peak xylose concentrations range from 45 to 75 mg/dl. Peak xylose concentrations in dogs suffering from bacterial overgrowth in the small intestine are 30 mg/dl. Peak xylose concentrations in the present study may indicate the presence of bacterial overgrowth in the small intestine, suggesting these calves were infected with multiple pathogens prior to coronavirus challenge. Necropsy of one calf revealed the presence of multiple pathogens, including coronavirus, E. coli, salmonella and clostridium. Salmonella was not a significant source of infection as there was no increase in rectal temperature. Another indicator of pathogen overload was a calf mortality rate of 24% in this study. Although calves were challenged with coronavirus after arrival, they were purchased from sale barns and exposed to many pathogens prior to arrival; infection with multiple pathogens may have severely disrupted the absorptive and secretory mechanisms of the intestinal tract in ways that were not compatible with the design of the study.

Decreased intestinal absorption and prolonged diarrhea in coronavirus challenged calves occurs as cells are sloughed off and replaced with immature cells unable to perform normal functions. These immature cells are redirected due to coronaviral infection to function only for viral production. Differences between treatments may have been more evident if calves were pretreated with concentrated serum protein fraction. Effects of viral challenge with *Cryptosporidium parvum* were ameliorated when colonic cells were pre-treated before viral challenge with TGF-, 1 versus cells that were treated with TGF-, 1 during or after challenge. Cells pretreated with TGF-, 1 were not as permeable as control cells and were able to better maintain their mucosal barrier.

Serum protein fraction used in the present study included elevated concentrations of IGF-I (1368 ng/ml), TGF-, 1 (765 ng/ml), TGF-, 2 (9 ng/ml) and IgG (19.7%) fractions. Levels of IGF-I found in first-milking colostrum range from 248 to 1850 ng/ml, while TFG-, 1 concentrations range from 12.4 to 42.6 ng/ml. The serum protein fractions were fed at 1% of BW, with total concentration of TGF-, 1, for example, fed at .2754 mg versus TGF-, 1 in colostrum fed at birth at .015336 mg for a calf with an average bodyweight of 36 kg. Immunoglobulin G may defend the intestine by preventing the attachment of organisms, reducing their ability to replicate and invade the gut. Treatment with immunoglobulins may also affect calves favorably if infection with other enteric pathogens occurs.

Although no significant differences were seen between treatments in the present study, many unforeseen factors may have affected these data. Further research needs to be conducted to clarify effects of growth factors in response to controlled damage to intestinal epithelium.

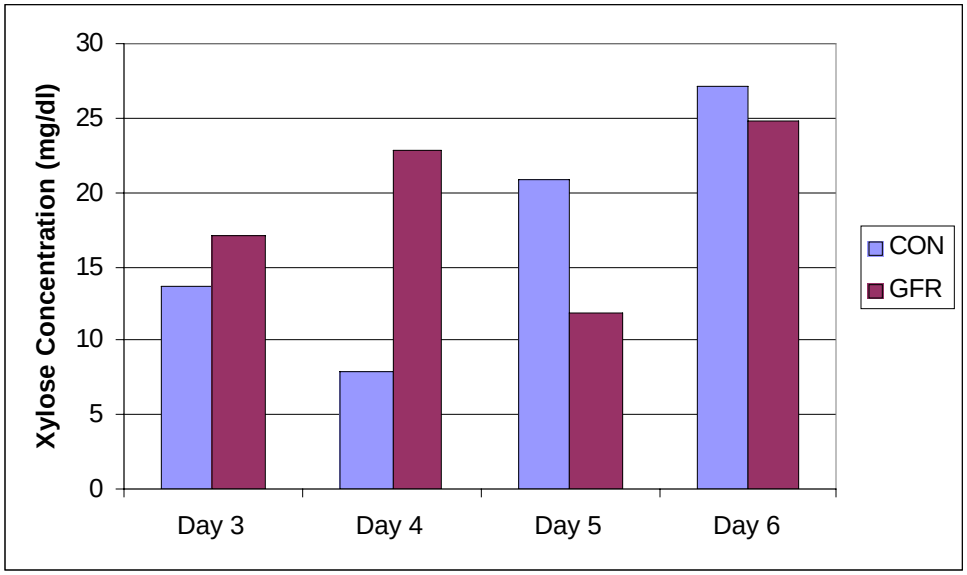

Figure 1. Changes in xylose concentrations (mg/dl) for calves grouped by day (using baseline values as a covariate).

Table 1. Ingredient composition of control oral rehydration solution[1].

Ingredient	mmol/L
Sodium	135.71
Chloride	88.01
Potassium	188.73
Dextrose	111.25
Maltodextrin	111.25
Glycine	40.06
Sodium Citrate	25.33
Calcium Lactate	1.92
Ascorbic Acid	2.90

[1] Merrick's, Inc., Union Center, WI

Table 2. Ingredient composition of plasma serum fraction with elevated levels of growth factors[2].

Ingredient	Concentration
Total protein	93.24%
Albumin	56.64%
IgG	19.70%
Moisture	3.36%
Ash	0.61%
Standard Platelet Count	600 CFU/g
Endotoxin	0.311 EU/g
TGF-,1	765 ng/g
TGF-,2	9 ng/g
IGF-1	1368 ng/g
Salmonella	negative

[2] APC, Inc. Ames, IA