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RESEARCH ARTICLES

Effect of animal plasma proteins on intestinal damage and recovery of neonatal pigs infected with rotavirus☆

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Abstract

Rotaviruses infect and elicit diarrhea in neonates of most mammalian species and cause 800,000 infant deaths a year. We used neonatal piglets to study the effects of dietary animal plasma proteins on intestinal health following rotavirus infection. Plasma protein contains a diverse mixture of functional components with biological activity and improves the health of animals challenged with other diarrhea-causing pathogens. In a 2×2 factorial design, we compared plasma protein- and soy protein-based diets in rotavirus-infected and noninfected piglets to determine if plasma protein reduced acute rotavirus intestinal damage or improved recovery. All infected animals shed rotavirus particles in their feces. Infected, plasma protein-fed piglets maintained growth rates similar to noninfected piglets in the first 3 days of infection; however, soy protein-fed piglets experienced reduced gains. Furthermore, infected, plasma protein-fed piglets showed no clinical signs of diarrhea. Infection reduced intestinal villus height and the villus height/crypt depth ratio by Day 3 of infection; however, reductions were not attenuated with dietary plasma protein. Infected, plasma protein-fed pigs maintained greater intestinal mucosa protein and estimated total lactase activity than infected, soy protein-fed piglets. Plasma proteins contain growth factors that may aid in rate of recovery as well as virus-binding proteins that may reduce infection pressure in the intestine. These data, combined with findings from other studies using plasma proteins in animal models of diarrhea, indicate the potential for using plasma proteins to improve the health of diarrheic neonates.

Keywords: Rotavirus; Piglet; Plasma proteins; Intestinal health

1. Introduction

Each year, approximately 2.5 million infants and children worldwide die from diarrheal disease [1]. Although mortality has decreased over the last 30 years, morbidity has largely remained unchanged [1,2]. A leading cause of severe diarrhea in both developed and developing countries is rotaviral enteritis accounting for 600,000–800,000 deaths a year [3]. In the United States, the cost of rotavirus-related hospitalizations for infants and young children exceeds $1 billion annually [4]. Rotaviruses infect many animals including birds, companion animal species and production animal species [5] with piglets commonly used as a model for human rotavirus infection [6,7].

Rotaviruses infect small intestine enterocytes and cause direct damage to the mucosal lining. Viral particles replicate within the enterocytes [8], lysing the infected cells causing villus blunting and atrophy [9]. A malabsorptive-type diarrhea follows [10] with depressed mucosal disaccharidases [11] and watery diarrhea [9] accompanied by dehydration. Overall, the loss of normal villus architecture results in decreased nutrient uptake that can potentially result in a failure to thrive.

Weaned piglets on commercial farms typically experience diarrhea after weaning, resulting in impaired growth performance and increased mortality. Plasma proteins are routinely fed to newly weaned piglets at low inclusion rates (i.e. 2–5%) to increase feed intake and improve growth performance [12,13]. Performance improved with increasing incorporation of plasma proteins, and the magnitude of response to feeding plasma proteins increased when pigs were housed in higher pathogen exposure [14]. These findings indicate that inclusion of dietary plasma

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Table 1
Composition and calculated analysis of the experimental diets

	Soy protein	Plasma protein
Ingredient (%)		
Soy protein isolate[a]	33.8	21.1
Plasma protein[b]	0.0	15.0
Lactose[c]	34.11	33.90
Sucrose	5.0	5.0
Soybean oil	14.81	14.57
Lysine	0.41	0.05
Histidine	0.065	0.0
Leucine	0.065	0.0
Valine	0.17	0.0
Methionine	0.28	0.28
Tryptophan	0.032	0.0
Threonine	0.155	0.0
Vitamins[d]	0.127	0.127
Minerals[e]	0.5	0.5
Calcium Carbonate	1.32	1.83
Dicalcium phosphate	4.33	3.41
NaCl	0.83	0.28
$MgSO_4$	0.05	0.05
K_2PO_4	0.45	0.45
Lysolecithin	1.0	1.0
Agglomeration agent	1.5	1.5
Xanthan gum	1.0	1.0
Calculated analysis		
ME (kcal/kg)	3860	3928
CP (%)	30.0	30.0
Fat (%)	15.0	15.0
Lactose (%)	34.4	34.2
Lysine (%)	2.11	2.17
Ca (%)	1.50	1.50
P (%)	1.03	1.03

Values are expressed on a dry-weight basis.

[a] Archer Daniels Midland (Champaign, IL).

[b] Proliant.

[c] Milk Specialties (Dundee, IL).

[d] Vitamin Premix (Milk Specialties) contained 33,000,000 IU/kg vitamin A, 6,600,000 IU/kg vitamin D_3, 55,000 IU/kg vitamin E, 257,400 mg/kg vitamin C, 29,983 mg/kg D-pantothenic acid, 33,069 mg/kg niacin, 8378 mg/kg riboflavin, 5115 mg/kg menadione, 66 mg/kg biotin, 44,000 mg/kg vitamin B_{12}, 2038 mg/kg thiamine, 3996 mg/kg vitamin B_6 and 2756 mg/kg folic acid.

[e] Mineral premix (Milk Specialties) contained 1.002% Ca, 0.549% P, 0.284% Na, 0.040% Cl, 2.024% K, 0.102% Mg, 20,000 mg/kg Fe, 200 mg/kg Co, 1850 mg/kg Cu, 400 mg/kg I, 5000 mg/kg Mn, 60 mg/kg Se and 23,500 mg/kg Zn.

proteins may improve animal well-being in health-challenging environments.

A recent report demonstrated that *Cryptosporidium*-infected calves fed with bovine serum concentrate produced less diarrheal volume and had reduced intestinal permeability resulting in improved rate of intestinal recovery [15]. Piglets fed with plasma proteins and challenged with *Escherichia coli* K88 recovered from diarrhea more quickly [16] and had reduced intestinal inflammation [17]. The improved growth performance of weaned piglets and the improved health of *Cryptosporidium*-infected calves and *E. coli*-infected piglets fed with plasma proteins lead us to examine using plasma proteins to improve the health of rotavirus-infected piglets. The objective of this study was to determine if dietary plasma proteins reduced rotavirus-induced intestinal damage in neonatal piglets or improved the recovery of the damaged intestinal tract.

2. Materials and methods

2.1. Animal care

All procedures involving animals were approved by the North Carolina State University Institutional Animal Care and Use Committee. In two replicates, sows ($n=5$ per replicate) were injected with 12.5 mg of $PGF_2\alpha$ (Dinoprost; Pharmacia Upjohn, Kalamazoo, MI) 2 days prior to the expected farrowing date to induce parturition. At farrowing, pigs were collected directly from the birth canal, cleaned with a 70% ethanol solution and were immediately relocated to a sanitized rearing facility. Pigs were housed individually in cages (length, 0.5 m; width, 0.3 m; and height, 0.4 m) within a temperature-controlled room (32°C) and trained to suckle from a cross-cut nipple. Pigs were offered diets ad libitum via a gravity flow feeding system, consisting of a bottle suspended above a cage with tubing connecting the bottle to the nipple [18]. For the first 24 h, a liquid colostrum diet (Protein Technology, Santa Rosa, CA) was fed to the piglets, beginning within 2 h of farrowing to provide passive immunity. All components of the feeding system were thoroughly cleaned twice per day with a liquid chlorinated detergent (DS Liquid: Command, Diversey, Wyandotte, MI) and cages were cleaned daily.

2.2. Experimental design

Pigs ($n=64$) were blocked by litter and randomly assigned to one of four treatments in a 2×2 factorial design ($n=16$ pigs per treatment). Pigs were assigned to different rooms according to future rotavirus infection (noninfected vs. rotavirus-infected) and to one of two experimental diets. The treatment diet contained 15% animal plasma proteins (AP-920, Proliant, Ankeny, IA) while the soy protein control diet contained no animal products other than lactose (Table 1). The diets were formulated to provide similar levels of energy, protein, fat, lactose and essential amino acids. Crystalline amino acids were added to the soy diet because of the differing amino acid profiles of the soy and plasma protein sources. Diets were reconstituted daily at 150 g/L of water (approximately 12% dry matter) and stored at 4°C. Fresh diet was provided four times daily (0800, 1300, 1800 and 2300 h) to minimize spoilage and to ensure ad libitum access to diets.

After 4 days of dietary treatment, pigs were infected with either 0 or 10^7 rotavirus particles according to previous infection assignment (noninfected vs. infected). The rotavirus inoculum utilized in this study was isolated from a commercial swine farm [19]. Rotavirus and sham inoculants were mixed in 20 ml of their respective diet and the pigs were gavaged using size 8F stomach tubes. Pigs were denied access to diet 6 h prior to infection and then refed their respective diet starting 3 h postgavaging and maintained on

treatment diets until the end of the experiment. Feces were visually assessed and assigned a consistency score on a daily basis by a single person blinded to dietary treatments. A score of 0, 1, 2 or 3 was recorded to indicate firm, soft but formed, runny or severe watery diarrhea, respectively. Pigs were rectally swabbed with a cotton-tipped applicator daily for the detection of rotavirus shedding.

Pigs were humanely euthanized either 3 or 14 days postinfection to collect intestinal tissue. Termination at 3 days postinfection was chosen to determine the effects of diet on reducing the intestinal damage from the rotavirus infection, and termination at 14 days postinfection was chosen to determine if dietary treatments affected the recovery from rotavirus infection. Briefly, the abdomen was opened and the gastrointestinal tract removed from the gastroesophageal junction to the distal end of the rectum. The jejunum and ileum were dissected from the duodenum, stomach and mesentery from the peritoneal inflection to the ileocecal junction. At approximately mid-ileum, two adjacent segments, one 3 cm in length and another segment 10 cm in length, were removed. Mucosa from the 10-cm intestinal segments was scraped, weighed, and homogenized in buffer (10 mM phosphate-buffered saline, pH 7.5) for lactase activity. The 3-cm intestinal segments were fixed (10% formalin, 70% ethanol, 5% acetic acid, 15% water) for histological analysis.

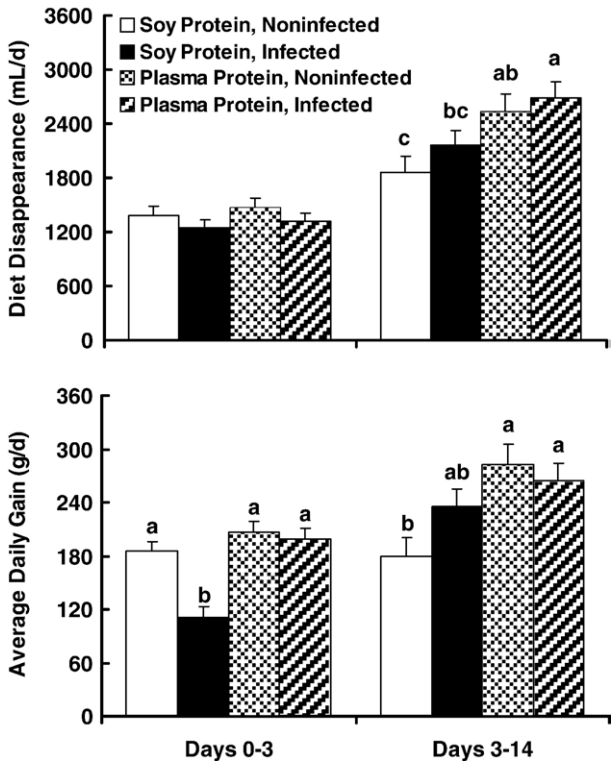

Fig. 1. Diet disappearance (ml/day; upper panel) and average daily gain (g/day; lower panel) of neonatal pigs fed with either a soy protein-based diet or a diet containing 15% plasma proteins 0–3 or 3–14 days postinfection with rotavirus. Pigs were infected with rotavirus or received excipient after 4 days of dietary treatment. Means within a time interval lacking a common superscript are significantly different ($P<.05$).

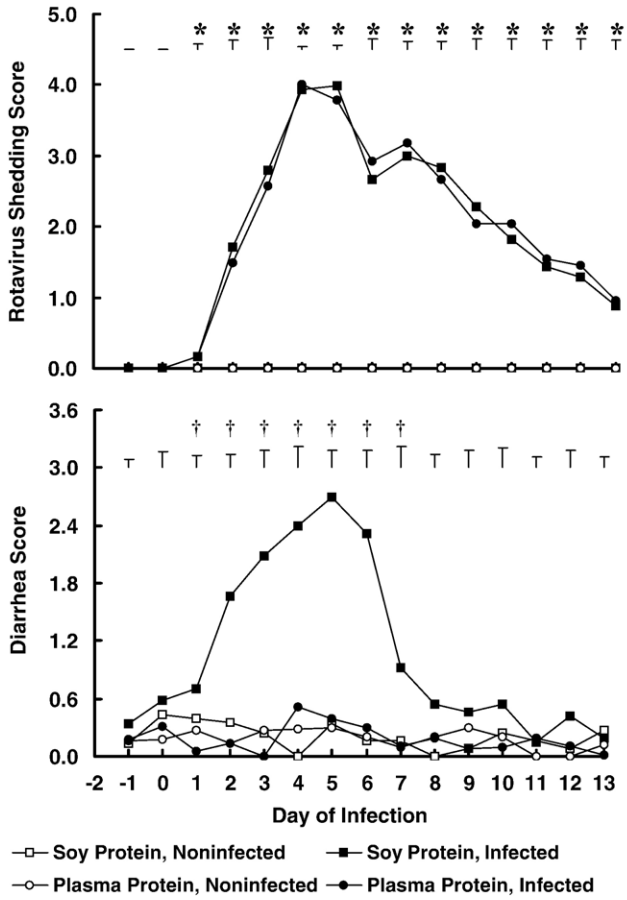

Fig. 2. Rotavirus shedding score (upper panel) and diarrhea score (lower panel) of neonatal pigs fed with either a soy protein-based diet or a diet containing 15% plasma proteins. Pigs were infected with rotavirus or received excipient after 4 days of dietary treatment. *$P<.05$; infected pigs were significantly different from noninfected pigs. †$P<.05$; soy protein-fed, infected pigs were significantly different from all other treatments. S.E.M. is indicated by error bars at the top of each panel.

2.3. Analytical methods

Rectal swabs collected for assessment of rotavirus shedding were placed in 2 ml of 10 mM phosphate buffer saline (pH 7.5) and briefly swirled. Five microliters of agglutination media was added to 10 μl of sample on a glass slide. Visual assessment of agglutination was measured after 4 min according to manufacturer's procedures (Wampole Laboratories, Cranbury, NJ). A score of 0, 1, 2 or 3 was assigned to samples with no agglutination, minimal, modest and high amount of agglutination, which represents extent of rotavirus shedding, respectively.

Intestinal segments for histological analysis were processed, embedded and stained with hematoxylin and eosin [20]. Morphometrics of the intestine segments were performed by one person using light microscopy with a computer-assisted morphometric system (BioScan Optimetric, BioScan, Edmonds, WA). The villus height and crypt depth of four well-oriented villi per sample were measured.

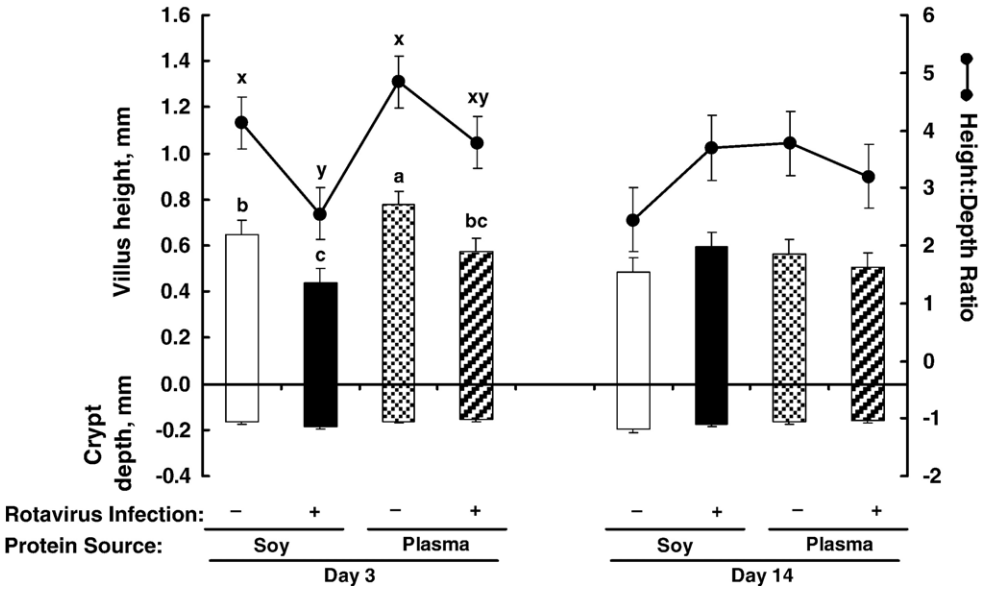

Fig. 3. Intestinal morphometrics of the ileum from neonatal pigs fed with either a soy protein-based diet or a diet containing 15% plasma proteins. Pigs were infected with rotavirus or received the excipient after 4 days of dietary treatment and ileum was sampled at either 3 or 14 days postinfection. Means within a time point lacking a common superscript are significantly different ($P<.05$).

Intestinal lactase activity was assayed according to methods described by Dahlqvist [21]. Protein content of the mucosa homogenate was assayed by Bradford [22].

2.4. Statistical analysis

Data were analyzed via two-way analysis of variance using the general linear models procedure of SAS (SAS Institute, Cary, NC). Pig was the experimental unit. The model included the main effects of dietary protein source and rotavirus infection as well as the interaction of the two effects. When a significant treatment effect was detected ($P<.05$), means were separated using a least significant difference test.

3. Results

During the first 3 days postinfection, no differences in feed intake were observed between diets ($P=.40$) or infection ($P=.13$) treatments (Fig. 1, upper panel). From Day 3 postinfection until termination of the experiment, pigs fed with plasma proteins consumed 30% more than pigs fed with the soy protein diet ($P=.002$), regardless of infection. Infected and noninfected pigs consumed similar amounts of diet from Days 3–14 ($P=.24$), regardless of diet. However, infected pigs fed with plasma proteins consumed a greater amount of diet ($P=.048$) than infected pigs fed with soy protein (Fig. 1, upper panel). During the initial interval following infection (0–3 days), there was a reduction in the average daily gain ($P<.001$) of infected pigs consuming the soy protein diet (Fig. 1, lower panel). These pigs achieved only 56% of the growth rate of the infected, plasma protein-fed pigs. From days 3 to 14, plasma protein-fed pigs gained more than soy protein-fed pigs regardless of infection status (272 vs. 208±15 g/day, $P=.004$), although there were no differences ($P=.30$) in average daily gain between infected plasma protein-fed pigs and infected soy protein-fed pigs.

No shedding of rotavirus was detected in any pigs prior to infection nor was shedding of rotavirus detected in

Table 2
Effect of plasma protein and rotavirus infection on ileum mucosal mass, protein mass and lactase activity at 3 or 14 days postinfection

	Soy protein		Plasma protein		S.E.M.
	Noninfected	Infected	Noninfected	Infected	
Day 3 of infection					
Mucosa (g/10 cm)[1]	2.84^{ab}	2.65^{ab}	3.09^{a}	2.45^{b}	0.19
Protein (mg/10 cm)[2]	109^{b}	71^{c}	137^{a}	98^{b}	9
Lactase activity [μmol/(min · g protein)]	45.8^{b}	40.0^{b}	85.0^{a}	58.9^{b}	9.4
Day 14 of infection					
Mucosa (g/10 cm)[1]	4.04	3.92	3.84	3.58	0.25
Protein (mg/10 cm)[2]	124	131	161	159	16
Lactase activity [μmol/(min · g protein)]	19.1^{b}	45.7^{a}	50.5^{a}	51.4^{a}	7.5

Values are means±S.E.M., $n=16$ per group. ^{a,b,c}Means lacking a common superscript are significantly different ($P<.05$).

[1] Wet weight of mucosa from a 10-cm section of the ileum.

[2] Protein content of mucosa from a 10-cm section of the ileum.

noninfected pigs for the duration of the study (Fig. 2, upper panel). Shedding of rotavirus was detected in infected pigs 24 h postinfection and increased to a maximum by Days 4 and 5 postinfection ($P<.001$), declining throughout the remainder of the study. Infected pigs shed similar amounts of virus regardless of dietary treatment ($P>.18$).

Diarrhea score was visually assessed at the same time on a daily basis. Infected, soy protein-fed pigs showed the greatest diarrhea scores (Fig. 2, lower panel). These pigs had significantly greater diarrhea scores ($P<.001$) within 1 day of infection and maintained greater scores through Day 7 of infection ($P<.007$) with peak diarrhea score on Day 5. Throughout the course of the experiment, infected, plasma protein-fed pigs did not demonstrate diarrhea scores different ($P>.10$) from noninfected pigs of either dietary treatment. By Day 8 of infection, diarrhea scores of infected, soy protein-fed pigs had returned to normal and were not different ($P=.08$) from those of the other treatment groups. On Day 3, plasma protein-fed pigs had greater fecal dry matter content than soy-fed pigs ($P<.001$; data not shown) regardless of infection.

The morphometrics of the ileum were examined 3 and 14 days postinfection to assess the structural status of the intestine. Infected pigs experienced reductions in villus height (0.506 vs. 0.713 ± 0.042 mm, $P<.002$) and the villus height/crypt depth ratio (3.17 vs. 4.49 ± 0.31, $P=.004$) 3 days postinfection (Fig. 3), regardless of diet. Plasma protein-fed pigs had greater villus height (0.674 vs. 0.544 ± 0.042 mm, $P=.03$) and greater height/depth ratios to infection (4.32 vs. 3.34 ± 0.31, $P=.03$) than soy protein-fed pigs. No differences in crypt depths were observed following 3 days of infection ($P=.43$), and no differences in villus height ($P=.68$), crypt depth ($P=.18$) or the height/depth ratio ($P=.52$) were observed 14 days postinfection.

We quantified the mucosa weight and protein content, as well as the lactase activity of the ileum after 3 and 14 days postinfection to assess the digestive capability of the intestine (Table 2). Infection reduced the weight of mucosa (2.55 vs. 2.96 ± 0.13 g, $P=.03$) and mucosal protein (85 vs. 123 ± 6 mg, $P<.001$) after 3 days of infection, regardless of diet. Infection also tended to reduce lactase-specific activity on Day 3 [49.4 vs. 65.4 ± 6.5 μmol/(min·g protein), $P=.083$]. Pigs fed with plasma proteins had greater mucosal protein (118 vs. 90 ± 6 mg, $P=.003$) and lactase activity than pigs fed with soy protein [72.0 vs. 42.9 ± 6.5 μmol/(min·g protein), $P=.003$], regardless of infection. Additionally, infected plasma protein-fed pigs maintained greater ($P=.03$) mucosal protein content than infected, soy-fed pigs (Table 2). No differences between treatments were observed in the biochemical status of the intestine following 14 days of infection, although noninfected soy protein-fed pigs had less lactase-specific activity compared to the other three treatments ($P<.01$). There were no differences in fecal digestibility determined using an inert marker (Co-EDTA) on Day 3 of infection; however, pigs fed with plasma proteins had reduced digestibility on Day 14 of infection ($P=.015$; data not shown).

4. Discussion

A rotavirus vaccine was developed in the mid-1999′s with the hope of preventing rotaviral infection; however, the vaccine was subsequently withdrawn from the market following reports of increased incidences of intussusception [23]. More recently, new vaccines have been developed that may offer the potential to prevent severe rotavirus diarrhea [24,25]. Efficacy and safety of two different vaccines have been examined, and the incidence of intussusception was not different from placebo [24,25]. Additional clinical trials in developing countries are required to confirm that children receiving vaccines in poor settings will respond in a similar manner due to factors that may reduce vaccine efficacy in children in these environments [26]. Vaccines for all species affected by rotavirus are not currently available, and we have sought to develop nutritional therapies to reduce the severity of rotaviral enteritis using a neonatal pig model. We have previously used this model of rotaviral enteritis to demonstrate a delay in recovery caused by malnutrition [6,7], and others used this model to test oral rehydration solutions [27] with little improvement in recovery or course of infection. We selected plasma protein inclusion as a possible nutritional therapy based on previous results indicating improvements in pig health under conditions of high pathogen exposure [14] and during the stressful period following weaning when pigs typically develop diarrhea [12]. Plasma protein is a diverse mixture of functional components consisting of immunoglobulins, growth factors, biologically active peptides and other factors with biological activity within the intestine, and in the current experiment, we observed significant improvements in the health of rotavirus-infected piglets during the acute stages of infection.

Infected piglets fed with plasma proteins grew significantly faster during the first three days of infection compared to infected, soy-fed piglets despite similar diet consumption between the groups. Early reports demonstrated that the growth effects observed in weaned pigs fed with plasma proteins could be related to improvements in palatability and feed intake [28]; however, more recent reports have shown plasma proteins improved the efficiency of dietary protein utilization through reductions in amino acid catabolism [13]. However, in our experiment, crystalline amino acids were added to the soy protein diet to match the amino acid profile of the plasma protein diet, minimizing the possibility that improvements in growth were related to improved amino acid supply by plasma protein. After 3 days of infection, noninfected, plasma protein-fed piglets consumed more and grew faster than noninfected, soy-fed piglets consistent with plasma protein's ability to stimulate feed intake and growth in weanling pigs [29]. These results would tend to indicate that the soy-based diet compromised

the nutritional status of the piglets; however, we have recently demonstrated superior growth in pigs fed with isolated soy protein compared with casein- or whey-based milk replacers [30], reducing this possibility. Infected, plasma protein-fed piglets tended to continue growing faster than infected soy-fed piglets; however, infected, plasma protein-fed piglets consumed a greater amount of diet during this time period, consistent with previous findings that plasma proteins increased daily feed intake and gain in pigs infected with *E. coli* [16,31,32].

Improvements in gut health and reductions in diarrhea incidence were also observed in plasma protein-fed piglets. As expected, infection reduced villus height and villus height/crypt depth ratio, although plasma protein-fed piglets experienced less dramatic reductions. Infected, plasma protein-fed piglets also had greater mucosal protein concentrations and estimated total lactase activity after 3 days of infection compared to soy-fed piglets. These data indicate that feeding plasma proteins to infected piglets during the acute stage of infection enabled them to maintain greater intestinal function than soy-fed pigs. Other studies also have shown that plasma proteins improve intestinal health during pathogen-induced diarrhea. Calves infected with cryptosporidia had greater villus surface area and lactase activity when fed with plasma proteins [15]. Furthermore, *E. coli*-infected piglets fed with plasma proteins had less histological damage to the intestinal epithelium [16]. Plasma proteins consistently improved gut health in these models, and the greater intestinal function throughout infection may have facilitated greater diet consumption maintaining improved growth rates.

The most dramatic result observed in the present study was the abolishment of diarrhea in rotavirus-infected pigs fed with plasma proteins. These piglets showed no signs of diarrhea despite rotavirus shedding scores similar to soy-fed piglets that showed significant diarrhea. However, shedding was not measured quantitatively and may have been different between diets, but not detectable by the method used. Reductions in diarrhea volume and severity have been previously observed in cryptosporidia-infected calves [15], *E. coli*-infected pigs [16] and calves [33], and *Staphylococcus aureus* enterotoxin B-infected rats [34] fed with plasma proteins. In these models of protozoal and bacterial diarrhea, and our viral model of diarrhea, the similar responses observed could relate to a common mechanism despite differences in pathogen and mode of diarrhea induction.

One possible explanation for the observed effects of feeding plasma proteins is neutralization of pathogens preventing infection. Nollet et al. [33,35] were unable to *E. coli* surface antigens in plasma protein preparations fed to pigs [35] and calves [33], instead attributing the health improvements observed to glycoproteins that block receptor binding of bacterial adhesions. Rotavirus uses sialic acid and integrin proteins as cell receptors [36], and the presence of plasma proteins similar in structure may reduce rotavirus binding and infection. In contrast, Owusu-Asiedu et al. [16] detected antibodies to *E. coli* antigens in plasma proteins fed to piglets and attributed reductions in diarrhea and mortality to their presence. Antibodies against rotavirus were likely present in the plasma proteins because rotavirus is endemic to the production environment. Several growth factors found in plasma proteins are candidates for stimulating intestinal growth, protein synthesis and repair following injury, including epidermal growth factor [37], insulin-like growth factors (IGFs) I and II, and transforming growth factor-β. To be effective, growth factors must first escape both highly acidic conditions and proteolytic cleavage. Milk casein or linkage to IGF binding protein-3 may protect IGF-I from proteolysis, and oral IGF-I does play a role in intestinal development of neonatal animals [38]. Alternatively, plasma protein administration may promote improved intestinal health by altering immune activation in response to infection. Dietary plasma proteins reduced T helper cell activation in Peyer's patches from rats infected with *S. aureus* and may involve the pro-inflammatory cytokines [34]. Weaning [39], *E. coli* [17] and rotavirus infection [40] result in an up-regulation of inflammatory cytokine production. Plasma protein has been shown to reduce the production of the pro-inflammatory cytokine tumor necrosis factor-α (TNF-α) during *E. coli* infection and TNF-α reduces the effectiveness of IGF-I in promoting protein synthesis [41]. Therefore, plasma protein administration may be enhancing mucosal protein synthesis by reducing inflammatory cytokine production and restoring the normal responsiveness of epithelial cells to IGF-I.

Rotavirus continues to be a devastating disease on a worldwide basis, although promising vaccines for infants are emerging. We have used neonatal piglets as a model for infants; however, rotaviruses infect many species for which effective vaccines are not available. From this study, it appears that plasma proteins are effective in reducing diarrhea, improving intestinal health, and maintaining growth with infection. In species lacking effective vaccines and in countries where infants may still experience significant rotavirus-induced diarrhea despite vaccination, plasma proteins may reduce infection severity and speed recovery. These findings are consistent with the work of others showing plasma proteins are effective at improving the health of animals afflicted with diarrhea caused by several different pathogens. Collectively, these data indicate the potential use of plasma proteins to improve the health of diarrheic neonates.

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