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ORAL DELIVERY OF L-ARGININE STIMULATES PROSTAGLANDIN-DEPENDENT SECRETORY DIARRHEA IN *C. PARVUM* INFECTED NEONATAL PIGLETS

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Abstract

Objectives—To determine if oral supplementation with L-arginine could augment nitric oxide (NO) synthesis and promote epithelial defense in neonatal piglets infected with *C. parvum*.

Methods—Neonatal piglets were fed a liquid milk replacer and on day 3 of age infected or not with 10^8 *C. parvum* oocysts and the milk replacer supplemented with L-arginine or L-alanine. Milk consumption, body weight, fecal consistency, and oocyst excretion were recorded daily. On day 3 post-infection, piglets were euthanized, and serum concentration of NO metabolites and histological severity of villous atrophy and epithelial infection were quantified. Sheets of ileal mucosa were mounted in Ussing chambers for measurement of barrier function (transepithelial resistance (TER) and permeability) and short-circuit current (I_{sc} ; an indirect measurement of Cl^- secretion in this tissue).

Results—*C. parvum* infected piglets had large numbers of epithelial parasites, villous atrophy, decreased barrier function, severe watery diarrhea, and failure to gain weight. L-arginine promoted synthesis of NO by infected piglets which was unaccompanied by improvement in severity of infection but rather promoted epithelial chloride secretion and diarrhea. Epithelial secretion by infected mucosa from L-arginine supplemented piglets was fully inhibited by the cyclooxygenase inhibitor indomethacin, indicating that prostaglandin synthesis was responsible for this effect.

Conclusions—Results of these studies demonstrate that provision of additional NO substrate in the form of L-arginine incites prostaglandin-dependent secretory diarrhea and does not promote epithelial defense or barrier function of *C. parvum* infected neonatal ileum.

Keywords

nitric oxide; enteral nutrition; epithelial defense; barrier function

The small intestine is lined by a single layer of epithelial cells that are responsible for nutrient and water absorption and serve as the first line of defense against a hostile intraluminal environment. The protozoal parasite, *Cryptosporidium parvum*, replicates within and depletes intestinal epithelial cells resulting in progressive villous atrophy, nutrient malabsorption, and severe diarrhea. Infection by *Cryptosporidium* is responsible for 6% of all diarrheal disease, 37.7% of reported recreational water-associated and 8.5% of drinking water-associated

outbreaks of gastroenteritis of known or suspected infectious etiology (1,2). Cryptosporidiosis is responsible for diarrhea in 7 and 12% of children in developed and developing countries respectively (1,3).

There are no consistently effective antimicrobial treatments or vaccines for *Cryptosporidium* infection. Recovery depends upon the timely induction of epithelial defense mechanisms and replacement of deleted epithelial cells with functionally mature intestinal epithelium. These mechanisms must outpace the life-threatening consequences of diarrheal dehydration and starvation. Recent studies suggest that insufficient numbers of epithelial cells, rather than their immaturity, may be responsible for malabsorption, as *C. parvum* infected epithelial cells appear able to express amino acid and glucose transporters at levels comparable to uninfected epithelial cells (4)(author's unpublished data). Accordingly, enteral therapies may represent feasible and practical avenues to promote epithelial defense and repair in *C. parvum* infection.

Infectious enteritis of a variety of causes is associated with an increase in intestinal synthesis of nitric oxide (5). In *C. parvum* infected neonatal piglets and mice, intestinal epithelial cells are induced to express nitric oxide synthase II (iNOS) in vivo (6,7). Knockout or pharmacological inhibition of iNOS activity results in significant increases in epithelial parasitism and oocyst excretion in *C. parvum* infection (6–8). Further, in vitro studies have shown a direct effect of nitric oxide on viability of *C. parvum* sporozoites (8).

Nitric oxide is synthesized exclusively from the amino acid L-arginine. In adult mammals, L-arginine is synthesized by the proximal convoluted tubules of the kidney. In the newborn, renal synthesis of L-arginine is only minimally developed (9) and L-arginine is synthesized instead by the intestinal epithelial cells (9–11). Injury to the intestinal epithelium, increased amino acid requirements for growth and repair, and poor bio-availability of L-arginine in milk (10, 12,13) may collectively predispose the neonate to L-arginine deficiency. In view of this, we have recently demonstrated significantly lower concentrations of L-arginine in intestinal mucosa of neonatal piglets with *C. parvum* infection compared to littermate controls (authors unpublished data). A significant role for L-arginine in neonatal gut defense is supported by studies where nitric oxide synthesis inhibitors extended the age to which neonatal mice remain susceptible to *C. parvum* infection (7) and identification of L-arginine deficiency as a risk factor for development of necrotizing enterocolitis (4,14–21). Accordingly, oral delivery of L-arginine may be useful to promote nitric oxide synthesis and epithelial defense in *C. parvum* infected neonates.

In addition to the anti-parasitic effects of nitric oxide, we have also demonstrated that nitric oxide stimulates the synthesis of endogenous prostaglandins by *C. parvum* infected neonatal intestine (22). Endogenous prostaglandins promote the maintenance of intestinal barrier function while at the same time inhibit neutral NaCl absorption and stimulate epithelial chloride secretion (23,24). Consequently, promoting the synthesis of nitric oxide in *C. parvum* infection may worsen the severity of secretory diarrhea. The purpose of the present study was to determine if oral supplementation with L-arginine could augment nitric oxide synthesis and promote epithelial defense in neonatal piglets infected with *C. parvum*. The neonatal piglet is a highly valuable and unique experimental model that fully recapitulates human cryptosporidial infection, the pathophysiology of which is not reproduced in traditional laboratory animals (25).

MATERIALS AND METHODS

Animals

Littermate piglets (n = 8) from the College of Agriculture and Life Sciences were removed from each of 4 sows at 24-hrs of age and transported to the College of Veterinary Medicine

where they were housed in control or infected isolation facilities. Piglets were fed a liquid milk replacer (non-medicated, Advance Liqui-Wean, Milk Specialties, Dundee, IL) for the first 2 days. On the third day, piglets were weighed and each litter evenly divided into L-arginine or L-alanine treatment groups on the basis of equal body weight. At this time, litters undergoing infection were administered an inoculum of 10^8 *C. parvum* oocysts (University of Arizona, Tucson, AZ) given by orogastric tube. Piglets were subsequently fed L-arginine or L-alanine supplemented liquid milk replacer for an additional 3 days. A total of 24 infected (12 L-arginine, 12 L-alanine) and 8 control (4 L-arginine, 4 L-alanine) piglets were studied. Piglets were sacrificed on day 3 post-infection, a time period shown to correspond to peak epithelial parasitism in this model (25). Piglets were anesthetized with ketamine (15 mg/kg) and xylazine (0.5 mg/kg) given intramuscularly and euthanasia was performed using sodium pentobarbital (200 mg/kg). Sections of ileum, beginning 5 cm above the ileocecal junction, were immediately removed for *ex vivo* studies. All protocols were approved by the North Carolina State University Institutional Animal Care and Use Committee.

L-arginine supplementation

Piglets were initially fed an un-supplemented diet of 68.4 g/kg/day of powdered milk replacer containing 8.52 g/kg dry matter of L-arginine. Given a 90.4% digestibility of L-arginine in milk (26), the basal milk replacer contained 7.7 g of L-arginine/kg dry matter which provided 0.53 g of L-arginine/kg/day. The concentration of L-arginine in the basal diet as fed was 7.55 mM. The estimated daily L-arginine requirement for a 1-week old piglet is 1.08 g/kg/day (27). The L-arginine-enriched diet was supplemented with 24 g of L-arginine/kg dry matter resulting in a total L-arginine delivery of 2.18 g/kg/day. The concentration of L-arginine in the supplemented diet as fed was 30 mM. The isonitrogenous diet was supplemented with 27.7 g of L-alanine/kg dry matter. Daily feedings were divided into 4 equal volumes given every 6-hours. Prior to each feeding, the volume of any unconsumed milk replacer remaining was measured and discarded. L-arginine (L-arginine hydrochloride, FW 174.2) and L-alanine (FW 89.1) were purchased from Ajinomoto AminoScience LLC (Raleigh, NC).

Body weight, fecal consistency, and oocyst excretion

On a daily basis, the body weight of each piglet was recorded and a thin fecal smear prepared by rectal insertion of a cotton-tipped applicator. Smears were stained by the Auramine-O technique (25). For each smear, total numbers of oocysts were counted within a 64-mm² grid using a fluorescence microscope (Zeiss; Welwyn Garden City, U.K.). Fecal consistency was recorded as formed (score of 1), semi-formed (score of 2) or liquid (score of 3).

Blood samples

Serum and citrate-anticoagulated blood was obtained from anesthetized piglets by cardiocentesis. After centrifugation at $2000 \times g$ for 5-min, plasma was immediately tested for ammonia concentration by enzyme assay using an automated clinical chemistry analyzer. Serum was frozen at -80°C and later tested for total NO₂ concentration after conversion of NO₃ to NO₂ by nitrate reductase (Griess Assay; Cayman Chemical, Ann Arbor, MI).

Barrier function studies

A 20-cm segment of ileum was removed from the abdomen and immersed in an oxygenated Ringer's solution. The intestine was opened by incision along the anti-mesenteric border and the seromuscular layers removed. Sections of mucosa were mounted in 1.13 cm² aperture Ussing chambers and bathed on both surfaces with a porcine Ringer's solution containing (in mM) 154.1 Na⁺, 6.3 K⁺, 1.2 Ca²⁺, 0.7 Mg²⁺, 137.3 Cl⁻, 24 HCO₃⁻, and 1.65 HPO₄²⁻. The serosal bathing solution contained 10 mM glucose and was balanced on the mucosal side with 10 mM mannitol. Solutions were oxygenated and circulated by gas-lift (95% O₂ 5% CO₂)

and maintained at 37°C by water-jacketed reservoirs. The spontaneous potential difference (PD) was measured using Ringer-agar bridges connected to calomel electrodes, and the PD was short-circuited through Ag-AgCl electrodes using a voltage clamp that corrected for fluid resistance. If the spontaneous PD was between -1.0 and 1.0 mV, tissues were current clamped at $\pm 100 \mu\text{A}$ for 5 s and the PD was recorded. Transepithelial electrical resistance (TER; $\Omega\cdot\text{cm}^2$) was calculated from the spontaneous PD and short circuit current (I_{sc}) which were recorded at 15-min intervals over a 120-min period.

TER is conventionally expressed on the basis of serosal surface area (i.e. the aperture of the Ussing chamber). As a consequence of villous atrophy, control and *C. parvum*-infected mucosa differ markedly in their mucosal surface area and accordingly, the surface area of paracellular pathway available per cm^2 of serosa for ion permeation. Thus, TER values were calculated as described by Collett et al (28) on the basis of total mucosal surface area of paracellular pathway available in each cm^2 of serosal area as approximated using Marcial's measurements of $0.2180 \mu\text{m}$ paracellular pathway/ μm^2 surface area of villus and $0.7680 \mu\text{m}$ paracellular pathway/ μm^2 surface area of crypt (29) and morphometric analysis of representative histological sections disclosing a 2.75 fold difference in the mucosal-to-serosal surface area ratio of muscle-stripped and mounted ileal mucosa from control and *C. parvum*-infected piglets (22,25). A detailed application of surface area effects on TER of porcine ileal mucosa has been published (30).

Isotopic flux studies of mucosal permeability were performed using ^3H -labeled mannitol ($0.2 \mu\text{Ci/ml}$ in $16.6 \mu\text{mol/ml}$ mannitol). Isotope was added to the mucosal reservoir 15-min after mounting the mucosa on the chamber. One 60-min flux period (from 60 to 120-min) was performed by taking paired samples from the serosal reservoir. Samples were counted in a liquid scintillation counter. Flux of mannitol from mucosa-to-serosa ($J_{m\rightarrow s}$) was calculated using standard equations.

Morphometric Analyses

Sections of ileum were fixed in formalin, paraffin-embedded, sectioned at $5\text{-}\mu\text{m}$, and stained with hematoxylin and eosin for examination by light microscopy. Three sections from each tissue were examined. Five well-oriented villi were selected by an examiner blinded to treatment category. Villi were considered well-oriented if the adjacent crypt lumen was patent to the level of the muscularis mucosa. Average villus height (from the crypt opening to the villus tip) and crypt depth were measured using an ocular micrometer and the percentage of denuded villus surface was calculated from linear measurements of epithelialized versus denuded villus perimeter. The total number of villous epithelial cells and total number of intracellular parasites were counted along the perimeter of each of the selected villi and averaged for each piglet.

Statistical Analysis

Data are reported as mean $\pm$ SE. For all analyses, $P \leq 0.05$ was considered significant. All data were tested for normality and equal variance using a statistical software package (SigmaStat, Jandel Scientific, San Rafael, CA). Normally distributed data were analyzed using a one-way ANOVA while non-parametric data were analyzed using a Kruskal-Wallis ANOVA on ranks or Mann-Whitney rank sum test. One L-arginine group piglet failed to become infected with *C. parvum*. Data from this piglet and its matching littermate (that received L-alanine) were excluded from analysis. Additionally, one L-arginine and one L-alanine treated piglet died or was euthanized due to severe infection, respectively. Only food consumption and body weight data were recorded for these piglets. Ussing chamber studies were limited to the number of animals that could simultaneously be accommodated on the day piglets were euthanized.

RESULTS

C. parvum infected piglets are L-arginine deficient

We have recently shown that ileal mucosal concentrations of L-arginine are significantly lower in *C. parvum* infected versus uninfected neonatal piglets (author's unpublished data). L-arginine plays an essential role in ammonia detoxification via the urea cycle. An increase in plasma ammonia concentration is considered to be a sensitive indicator of L-arginine deficiency in human infants and piglets (31–33). Therefore, plasma ammonia concentrations were measured in control and *C. parvum* infected piglets as a biochemical indicator of L-arginine deficiency. In infected piglets, plasma ammonia concentrations were significantly higher than that of uninfected animals (μM ammonia = 27 ± 3.6 uninfected control ($n = 4$); 46.3 ± 7 *C. parvum* ($n = 7$), $*P = 0.05$ One-way ANOVA).

C. parvum infection results in decreased milk consumption and weight loss

Piglets with *C. parvum* consumed significantly less milk replacer at the time of peak infection compared to uninfected piglets (average volume $\pm$ SE/pig/day = 1000 ± 0 ml control ($n = 8$), 912 ± 17 ml infected ($n = 24$); $***P = 0.001$, ANOVA on Ranks). Piglets receiving L-arginine consumed a similar volume of milk than did those receiving L-alanine (average volume/pig/day = 933 ± 19 ml L-arginine ($n = 12$), 891 ± 49 ml L-alanine ($n = 12$). In contrast to uninfected piglets, piglets infected with *C. parvum* failed to gain body weight. Body weight did not differ between infected piglets receiving L-arginine or L-alanine supplementation (Figure 1).

Oral L-arginine increases synthesis of nitric oxide in C. parvum infected piglets

To determine if oral delivery of L-arginine could promote synthesis of nitric oxide in *C. parvum* infected piglets, nitric oxide metabolites (NO_2 and NO_3) were measured in serum samples obtained from L-arginine and L-alanine treated control and infected piglets at the time of peak infection (Figure 2). Consistent with our prior studies (22), nitric oxide synthesis was significantly greater in *C. parvum* infected versus uninfected control piglets. Synthesis of nitric oxide was further increased in piglets supplemented with L-arginine but not L-alanine; although the difference was not statistically significant.

L-arginine worsens diarrhea severity in C. parvum infected piglets

Beginning on day 1 of infection, fecal consistency scores were significantly higher for piglets infected with *C. parvum* compared to uninfected piglets and averaged in consistency between semi-formed and liquid. At the time of peak infection (day 3), diarrhea was significantly more severe in piglets receiving L-arginine (liquid in all piglets) than for those given L-alanine. Amino acid supplementation had no effect on fecal consistency of uninfected piglets (Figure 3).

Oral L-arginine does not ameliorate epithelial infection or oocyst excretion by C. parvum infected piglets

We have previously shown that administration of a selective iNOS inhibitor to piglets infected with *C. parvum* inhibits nitric oxide formation and exacerbates epithelial parasitism and oocyst excretion (6). Having demonstrated that oral L-arginine is capable of increasing nitric oxide synthesis in *C. parvum* infected piglets, we sought to determine whether supplementation with L-arginine would ameliorate the severity of epithelial parasitism. When *C. parvum* infected piglets treated with L-arginine or L-alanine were directly compared, there were no statistically significant differences in severity of epithelial cell loss (villous atrophy), epithelial parasitism, or fecal excretion of oocysts. When infected piglets were compared to their treatment-matched controls however, L-arginine and not L-alanine was associated with significantly more severe epithelial cell loss and villous atrophy (Table 1).

Treatment of *C. parvum* infected piglets with oral L-arginine increases epithelial secretion by ileal mucosa

To determine if more severe diarrhea in L-arginine-treated piglets could be attributed to increased epithelial permeability or stimulation of epithelial secretion, sheets of ileal mucosa from uninfected and *C. parvum* infected piglets treated with L-arginine or L-alanine were mounted in Ussing chambers for measurement of transepithelial electrical resistance (TER), mucosal-serosal flux of ^3H -labeled mannitol, and measurement of short-circuit current (I_{sc}). Detailed isotopic flux studies in neonatal porcine ileum have previously demonstrated that I_{sc} is attributable to epithelial chloride secretion in this tissue (23). TER and flux of ^3H -labeled mannitol demonstrated significantly greater permeability of *C. parvum* infected compared to uninfected mucosa (Figure 4). Chloride secretion (indirectly measured as I_{sc}) was significantly greater for mucosa from infected piglets given L-arginine compared to those treated with L-alanine or their uninfected controls (Figure 5).

L-arginine-stimulated epithelial secretion by *C. parvum* infected ileum is mediated by prostaglandins

We have previously shown that synthesis of prostaglandins by ileal mucosa is significantly increased in *C. parvum* infection and can be attributed to stimulation of cyclooxygenase by nitric oxide derived from iNOS (22). To determine if the epithelial secretion promoted by treatment of *C. parvum* infected piglets with L-arginine was attributable to prostaglandin synthesis, we examined the ability of indomethacin to inhibit the increase in I_{sc} observed in mucosa from L-arginine-treated piglets. Incubation of infected ileal mucosa ex vivo with indomethacin ($5 \times 10^{-6} \text{ M}$) abolished the rise in I_{sc} observed in L-arginine-treated piglets while having no effect on ileal mucosa from piglets receiving L-alanine (Figure 6).

DISCUSSION

Following infection of neonatal piglets with *C. parvum*, large numbers of parasites accumulated in villous epithelial cells resulting in villous atrophy, increased transepithelial permeability, severe watery diarrhea, and failure to gain body weight. Supplementation of neonatal piglets with L-arginine increased the synthesis of nitric oxide in *C. parvum* infection as indirectly measured by the concentration of nitric oxide metabolites ($\text{NO}_2 + \text{NO}_3$) in serum. The increase in nitric oxide measured was not statistically significant, perhaps due to the large variation observed among piglets receiving L-arginine. Supplementation with isonitrogenous amounts of L-alanine did not increase nitric oxide synthesis, arguing against any alternate nitrogen source for NO_2 or NO_3 formation in the infection. Similarly, L-arginine did not promote nitric oxide synthesis by uninfected piglets as is consistent with absence of nitric oxide synthase induction in these animals (6,22).

While prior studies in *C. parvum* infected piglets have demonstrated that inhibition of iNOS exacerbates infection (6), supplementation with L-arginine did not attenuate the severity of epithelial infection when administered to piglets in the present study. These results suggest that endogenous levels of nitric oxide are sufficient for optimal epithelial defense in *C. parvum* infection. Provision of insufficient amounts of L-arginine to piglets is unlikely to account for this finding. We have recently demonstrated that intestinal epithelial cells are fully capable of L-arginine uptake from the lumen of the intestine in *C. parvum* infection (unpublished data). Further, in prior studies as little as 0.24 g/kg/day of supplemental L-arginine increased plasma concentrations by 84% when given to milk-fed young pigs (34) and 0.261 g/kg/day of additional L-arginine increased plasma concentrations and abrogated necrotizing enterocolitis in pre-term human infants (14,18). In this study, piglets were supplemented with 1.65 g/kg/day of additional L-arginine. Despite a significant decline in milk consumption by *C. parvum* infected piglets, the amount of L-arginine supplement consumed

can be calculated to average 1.5 g/kg/day. Our decision to allow piglets to feed voluntarily rather than forcibly was based on prior observations that handling stress may promote cortisol-induced arginase expression by the intestinal epithelium, resulting in competition between arginase and iNOS for catabolism of L-arginine (35). We cannot rule out the possibility that L-arginine may be effective in ameliorating epithelial infection when administered to animals with less severe *C. parvum* infection than is reported here.

Administration of L-arginine to *C. parvum* infected piglets in this study had the undesired effect of worsening the severity of diarrhea. Diarrhea could not be attributed to differences in severity of villous atrophy, number of epithelial parasites, or epithelial permeability between L-arginine and L-alanine treated piglets. Ileal mucosa from *C. parvum* infected piglets treated with L-arginine was uniquely characterized by a significant increase in short-circuit current, a measurement reflective of epithelial Cl^- secretion in this tissue (23,36–38). Electrogenic absorption of L-arginine is unlikely to account for the short-circuit current measured in ileal mucosa from *C. parvum* infected piglets supplemented with this amino acid. We have been unable to demonstrate any direct effect of L-arginine on I_{sc} when added to the luminal reservoir of uninfected or *C. parvum* infected mucosa while mounted in Ussing chambers. Further, L-arginine was not present at the time I_{sc} measurements were performed on mucosa in the present study. Lastly, treatment with L-arginine did not increase I_{sc} of intestinal mucosa from uninfected piglets wherein we have demonstrated identical uptake mechanisms for L-arginine, only 50% of which are sodium dependent (unpublished data).

Endogenous prostaglandins serve as the primary mediators of Cl^- secretion in *C. parvum* infection by exerting direct effects on the intestinal epithelium and indirectly by stimulation of enteric nerves (23,36,37). We recently demonstrated that nitric oxide is the proximal mediator of prostaglandin synthesis in *C. parvum* infection and that induction of nitric oxide synthase gives rise to an increase in prostaglandin synthesis (22). Therefore, increasing nitric oxide formation by administration of L-arginine likely promoted secretory diarrhea by stimulating the further synthesis of endogenous prostaglandins. In support of this, enhanced Cl^- secretion by ileal mucosa from L-arginine-treated piglets was fully inhibited by treatment with the cyclooxygenase inhibitor indomethacin. Further, L-arginine did not promote epithelial secretion or diarrhea in uninfected piglets where inducible nitric oxide synthesis is absent. While lesser amounts of L-arginine may not stimulate epithelial secretion, they are also unlikely to impart attributes of epithelial defense that were lacking at higher doses. We have previously shown that prostaglandins help to maintain epithelial barrier function in *C. parvum* infected ileum (22). It is not surprising however, that L-arginine did not enhance barrier function in this study as treatment of infected ileum with prostaglandins *ex vivo* does not stimulate greater amounts of barrier function than are observed *in vivo* (22).

Highly diverse effects of nitric oxide on intestinal transport have been reported (39), ranging from inhibition (40) to stimulation of epithelial secretion as reported here. Such varying results may be attributed differences in location and quantity of nitric oxide synthesis, type and magnitude of inflammation, presence and activity of nitric oxide responsive pathways such as the cyclooxygenases, animal species, method of injury, and experimental conditions. Taken together with prior work on nitric oxide in *C. parvum* infection (6–8,22), results of these studies demonstrate that endogenous levels of nitric oxide are sufficient for promoting epithelial defense and barrier function in *C. parvum* infected neonatal ileum and that provision of additional nitric oxide substrate in the form of L-arginine incites prostaglandin-dependent secretory diarrhea.

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Fig. 1.

Percent of initial body weight of uninfected control and *C. parvum* infected piglets. Piglets were infected or not with *C. parvum* and milk replacer was supplemented with L-arginine or L-alanine beginning on day 0. Values are average $\pm$ SE. Numbers in parenthesis indicate number of piglets. *** $P < 0.001$ and ** $P < 0.01$ versus uninfected controls at time points shown (One-way ANOVA). * $P < 0.05$ between control + L-arginine and control + L-alanine at the time point shown (One-way ANOVA).

Fig. 2.

Concentration of nitrite + nitrate in the serum of uninfected control and *C. parvum* infected piglets at the time of peak infection. Piglets received either basal milk replacer alone (no treatment) or milk replacer supplemented with L-arginine or isonitrogenous amounts of L-alanine. ** $P = 0.003$ versus uninfected control (ANOVA on Ranks). n = number of piglets.

Fig. 3.

Fecal consistency score (average $\pm$ SE) of uninfected control and *C. parvum* infected piglets.

Piglets were infected or not with *C. parvum* and milk replacer was supplemented with L-arginine or L-alanine beginning on day 0. Numbers in parenthesis indicate number of piglets.

*** $P < 0.001$, ** $P < 0.01$ and * $P < 0.05$ versus uninfected control piglets at time points shown.

* $P < 0.05$ between infected + L-arginine and infected + L-alanine at the 3 day time point (Mann-Whitney Rank Sum Test).

Fig. 4.

Transepithelial electrical resistance (TER) and mucosal-to-serosal flux (J_{m-s}) of ^3H -labeled mannitol across control and *C. parvum* infected ileal mucosa from piglets supplemented with L-arginine or L-alanine beginning at the time of infection (day 0). Animals were euthanized on day 3 of infection at which time mucosa from each piglet was mounted in Ussing chambers. TER of infected piglets was significantly less than that of controls at each time point (* $P < 0.05$, one-way ANOVA). Flux of ^3H -labeled mannitol was significantly greater in infected versus control piglets (** $P < 0.01$, one-way ANOVA). Numbers in parenthesis indicate number of piglets. Values are average $\pm$ SE.

Fig. 5.

Short-circuit current measured in ileal mucosa from control and *C. parvum* infected piglets that were supplemented with L-arginine or L-alanine beginning at the time of infection (day 0). Animals were euthanized on day 3 of infection at which time mucosa from each piglet was mounted in Ussing chambers. Where indicated the non-selective cyclooxygenase inhibitor indomethacin (INDO, 5×10^{-6} M) was added to the mucosal and serosal reservoir to block endogenous prostaglandin synthesis. Measurements shown were recorded 75-minutes after addition of INDO at which time maximal differences between treatments were observed. For each bar, number of piglets is indicated. Values are mean $\pm$ SE. * $P < 0.05$ one-way ANOVA.