Acidosis and Lipopolysaccharide from *Escherichia coli* B:055 Cause Hyperpermeability of Rumen and Colon Tissues

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ABSTRACT

The objective of the present investigation was to evaluate the effects of acidic pH of the perfusate and presence of lipopolysaccharide (LPS) on permeability of rumen and colon mucosal tissues to mannitol and LPS using the Ussing chamber system. Rumen and colon tissues ($n = 8$), obtained from slaughtered feedlot steers, were tested for changes in permeability to ^3H -mannitol under pH of 4.5, 5.5, and 6.5 for rumen and at 5.5, 6.5, and 7.4 for colon, with or without LPS from *Escherichia coli* B:055 at 500 $\mu\text{g/mL}$. The ^3H -Mannitol was added at 10 μL (525.4 GBq/mmol) on the mucosal side of the Ussing chamber to detect changes in permeability, and 4 samples were taken at 20, 25, 30, and 35 min from the serosal side. Permeability of rumen and colon mucosa to ^3H -mannitol increased 6- and 5-fold, respectively, at acidic pH values of 4.5 and 5.5 and in the presence of 500 $\mu\text{g/mL}$ of LPS. In contrast, LPS did not affect rumen and colon permeability at pH that ranged from 5.5 and 7.4. Translocation of LPS across the rumen and colon mucosa of cattle was not pH dependent. The LPS translocated through these tissues if present at the mucosal side. In conclusion, the permeability of rumen and colon tissues to ^3H -mannitol increased in presence of LPS and under acidic pH, whereas LPS permeated through mucosal tissues independently of the pH of the perfusate. Further research is warranted to understand the mechanism(s) by which acidic pH of the rumen digesta and presence of LPS make rumen and colon tissues “leaky”.

Key words: cattle, acidosis, lipopolysaccharide, mucosal permeability

INTRODUCTION

Feeding dairy cows diets containing high proportions of grain is associated with a rapid decline in ruminal

pH (i.e., acidosis) as well as alterations in the microbial ecology (Keunen et al., 2002). Based on the degree and the length of time at which ruminal pH remains below a certain threshold, 2 conditions are identified in dairy cows as acute or subacute ruminal acidosis (ARA or SARA, respectively). The critical thresholds for diagnosis of ARA or SARA are defined as ruminal pH values of <5.0 or <5.6 , respectively (Nagaraja and Town, 1990). Cows affected by acute ruminal acidosis develop symptoms that include decreased DMI, loss of BW, diarrhea, and lameness (Nocek, 1997). Other events associated with SARA or ARA are decreased ruminal motility, stasis, rumenitis, and hyperkeratosis (Dirksen et al., 1984). The mechanism(s) by which rumen acidosis affects general health and performance of cows is not clear; however, rapid fermentation of starch and the release of vast amounts of VFA and lactic acid have been implicated in development of clinical pathology.

Feeding cattle high-grain diets is also associated with 18- to 20-fold increase in the amount of endotoxin in the rumen fluid (Nagaraja et al., 1978a). There are indications that endotoxin translocates into the bloodstream and causes a variety of metabolic and immunologic alterations to the host (Andersen et al., 1994). Moreover, endotoxin plays a role in several metabolic disorders in cattle such as laminitis, abomasal displacement, fatty liver, and sudden death syndrome (Ametaj et al., 2005). The precise mechanism(s) and the favorable conditions that facilitate translocation of endotoxin into the blood circulation are not known, and attempts to measure endotoxin in the systemic circulation have not been very fruitful (Andersen et al., 1994; Gozho et al., 2007).

Because acidic environments and the presence of endotoxin render the mucosal epithelium more permeable and susceptible to apoptosis (Chin et al., 2006), we hypothesized that exposing rumen and colon tissues of cattle to acidotic pH and LPS will alter their permeability and lead to translocation of LPS through these tissues. To test our hypothesis we conducted an in vitro experiment in an Ussing chamber system.

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Table 1. Reagents¹ and final concentration in the amino acid solution

Reagent	Final concentration (mmol)	Quantity (g/L)
Lactobionate	20	7.1660
Glutamate	20	2.9420
Aspartic acid	20	2.6620
Arginine	10	1.7420
Proline	5	0.5755
Glycine	10	0.7507
Cysteine	5	0.6060
Asparagine	10	1.5010
Threonine	10	1.1910
Lysine	10	1.8260
Methionine	5	0.7460
Serine	10	1.0510
Leucine	5	0.6560
Isoleucine	5	0.6560
Valine	10	1.1710
Histidine	5	0.7760
Tyrosine	1	0.1812
Tryptophan	1	0.2042
Ornithine	5	0.8430
Trolox	1	0.2500
3-Aminobenzamide	1	0.1360
HO-butyrat	3	0.3783
Glucose	20	3.6040
N,N-Bis(2-hydroxyethyl)taurine	15	3.1980
Glutamine	35	5.1130
Adenosine	5	1.3360
Allopurinol	1	0.1361
Hydroxyethyl starch	5 g/dL	50.0000

¹The pH of the solution was adjusted to 7.40 ± 0.01 using 2 M NaOH.

MATERIALS AND METHODS

Animals and Treatments

Sample Collection and Preparation. Rumen and colon samples for the experiment were collected from an Edmonton-based abattoir (Edmonton Custom Packers, Edmonton, Alberta, Canada). Colon samples were selected for the experiment because a large number of gram-negative bacteria and especially *Escherichia coli* harbor that section of the gastrointestinal tract of ruminant animals (Grauke et al., 2002). Within 10 min after slaughter of healthy feedlot steers ($n = 16$), a 15-cm² area of rumen tissue or a 15-cm length of colon were excised from the gastrointestinal tract of each animal, washed with cold Ringer's lactate (Sigma-Aldrich Canada Ltd., Oakville, Ontario, Canada) solution, and stored in an enriched amino acid solution (Salehi et al., 2007) until processing and mounting the tissues in the Ussing chamber. Composition of the amino acid solution and Ringer's lactate are given in Tables 1 and 2, respectively. The amino acid-based solution is an intestinal-specific preservation solution developed experimentally for the cold storage of intestine (Salehi et al., 2007). Samples were carried to the laboratory in the cold amino acid solution (4 to -5°C) in a cooler and

Table 2. Reagents¹ and their final concentrations in Ringer's lactate solution

Compound	Final concentration (mmol)	Quantity (g/2 L)
MgCl ₂ ·6H ₂ O	114.00	0.447
CaCl ₂ ·2H ₂ O	5.00	0.367
NaCl	1.65	13.320
KCl	0.30	0.750
NaHCO ₃	1.10	4.200
Na ₂ HPO ₄	1.25	0.468
NaH ₂ PO ₄ ·2H ₂ O	25.00	0.094
³ H-Mannitol	1.00	0.364

¹The reagents were added in the order listed and brought to final volume with doubly distilled H₂O and pH 7.4 using HCl or NaOH.

during transportation the samples were aerated with a syringe to facilitate respiration of the tissues. On reaching the laboratory, samples were prepared for mounting in an Ussing chamber on a glass surface which was kept cold by ice underneath. The serosal and muscular layers were peeled off carefully from both rumen and colon samples, and the maximum possible fibrous tissue was removed from the mucosa without injuring the tissue. Rumen ($n = 8$) and colon ($n = 8$) samples were collected from 16 different animals. One rumen or 1 colon sample was run on a certain day. The rumen or colon tissue was cut into 6 squares (approximately 2 cm²) for mounting in 6 different Ussing chambers (3 control and 3 treatments) exposing an area of 1.76 cm². The time from collection to mounting of tissues in the chambers was approximately 40 min.

Ussing Chamber Experiment. After turning on the water heater and assembling the lucite chambers between the supporting screws, 10 mL of Ringer's lactate at pH 7.4 was added to both reservoirs of the Ussing chamber. The quantity of each reagent for preparation of Ringer's lactate is shown in Table 2. Any air bubbles in the lucite chamber were removed by adjusting the leads. To both reservoirs of Ussing chamber, 200 μL of a 1-mmol glucose solution was added and chambers were connected to a 95% O₂/5% CO₂ airlift. Once the Ringer's lactate in the system attained a temperature of 39°C, the resistance button was adjusted to bring the fluid resistance to zero. The instrument was then switched to standby and the tubular ends of the lucite chambers were clamped on both the serosal and the mucosal reservoirs, and the right half of the chamber was removed. The prepared colon or rumen tissue was mounted on the pins of the chamber with the mucosal side in contact with the left lucite chamber and the chambers were then reassembled. Once the rumen or colon tissues were mounted on all the 6 Ussing chambers (3 controls and 3 treatments), the Ringer's lactate in the mucosal side of the chamber was drained and

replaced with Ringer's lactate and pH was adjusted to 4.5, 5.5, or 6.5 for rumen samples and 5.5, 6.5, and 7.4 for colon samples. After adjusting the pH, 10 μ L of 3 H-mannitol (525.4 GBq/mmol; PerkinElmer, Woodbridge, Ontario, Canada) and 500 μ g/mL of LPS from *E. coli* B:055 (Sigma-Aldrich Canada Ltd.) were added to the solution on the mucosal side of the chambers, on the treatment samples, to evaluate permeability to 3 H-mannitol and LPS. The amount of LPS used was chosen based on the amount of endotoxin in the rumen fluid of cattle fed all-grain diets (86 to 860 μ g/mL of LPS; Nagaraja et al., 1978b). The system was allowed to equilibrate for 15 min, and at 15 min 2 samples (radioactive; 100 μ L each) were taken from the mucosal side of the chambers to detect the initial concentration of 3 H-mannitol. At time 20, 25, 30, and 35 min, 1-mL and 200- μ L samples (nonradioactive) were taken separately from the serosal compartment for detection of 3 H-labeled mannitol and LPS. At the end of 40 min, another 2 fluxes (100 μ L each) were taken from the mucosal side of the chambers to detect the available concentration of 3 H-mannitol in the mucosal side after translocation into the serosal side. Although multiple samples were obtained at different time points, results presented are the average of all time points. After each sampling, measurements were made to determine the isoelectric current and the potential difference.

Determination of 3 H-Mannitol

Four milliliters of scintillation fluid was added to the vials for detection of 3 H-mannitol. The amount of radiation was measured as counts per minute in a liquid scintillation counter (Beckman Instruments, Irvine, CA). The quantity of translocated 3 H-mannitol was calculated from the difference between the counts per minute of radioactive and nonradioactive fluxes.

Determination of LPS

Lipopolysaccharide from *E. coli* B:055 in the serosal side of the chamber was quantified indirectly by determining the amounts of C₁₂-lauric and C₁₄-myristic fatty acids in the solution (Silipo et al., 2002). The fatty acid composition of LPS from *E. coli* is described by Datta and Basu (1999). For measurement of LPS, 100 μ L of the sample was freeze-dried. The freeze-dried samples were methylated by adding 1,000 μ L of methanolic HCl 3 N (Supelco, Bellefonte, PA). The samples were then vortexed before placing them in a water bath at 50°C and shaken every 5 min for 30 min. Methylated fatty acids were extracted by adding 50 μ L of H₂O and 3 mL of hexane and shaking vigorously for 20 s. The top hexane portion was then removed using a disposable

Pasteur pipette (Fisher Scientific, Fair Lawn, NJ) and dried under liquid nitrogen. The dried fatty acids were again dissolved in 150 μ L of hexane containing internal standard (2 mg C₁₇ in 1,000 μ L of hexane) and injected into 50 mm $\times$ 0.25 mm film thickness capillary column (Supelco) in a Varian 3400 gas chromatography equipped with Varian 8100 autosampler (Varian, Canada, Inc. Mississauga, Ontario, Canada). Helium was used as the carrier gas at a rate of 1.5 mL/min. Injector temperature was programmed from 50°C to 230°C at 150°C/min with a run time of 36 min. Detector temperature was set at 230°C, and peak area integration for fatty acids were made using Galaxy software (Varian Inc., Walnut Creek, CA).

Statistical Analyses

Data were subjected to statistical analysis by MIXED procedure of SAS Institute Inc. (1989) to determine the effects of presence of LPS and acidic pH on permeability to 3 H-mannitol and translocation of LPS through rumen and colon tissues, using the following model:

$$Y_{ijk} = \mu + T_i + P_j + TP_{ij} + A_k + e_{ijk},$$

where μ is the population mean; T_i is the fixed effect of treatment i where $i = 1, 2$; and P_j is the fixed effect of pH j where $j = 1, 2$, and 3; TP_{ij} is the effect of treatment $\times$ pH interaction; A_k is the random effect of sample k where $k = 1$ to 8; and e_{ijk} is the residual error. The PDIF option was used in each of the comparisons. Significance was declared at $P < 0.05$.

RESULTS

Permeability of Rumen and Colon Tissues to 3 H-Mannitol

Acidic pH with the presence of LPS increased permeability of rumen tissue to 3 H-mannitol ($P < 0.05$; Figure 1). Presence of LPS increased permeability of rumen tissue to 3 H-mannitol with a permeability of 11.7 nmol/cm²/h compared with 4.9 nmol/cm²/h in the controls. An interaction between LPS and pH was obtained with respect to permeability of rumen tissue to 3 H-mannitol ($P < 0.05$) at the lowest pH value of the perfusate. Permeability increased by more than 6-fold at a pH of 4.5 in the presence of LPS (4.10 vs. 25.09 nmol/cm²/h). But, presence of LPS did not affect permeability of rumen tissue to 3 H-mannitol at pH values of 5.5 and 6.5.

At pH 5.5 in the presence of LPS there was increased permeability of colon tissues to 3 H-mannitol (Figure 2; $P < 0.05$). The interaction between pH and LPS was evident, with increased permeability to 3 H-mannitol at the lowest pH value ($P < 0.01$). At pH values of 5.5, the

Figure 1. Permeability of rumen tissue obtained from steers to ³H-mannitol under different pH and in presence (■) or absence (□) of LPS from *Escherichia coli* B:055 in an Ussing chamber (Trt = treatment; pH = pH value of perfusate; Trt × pH = treatment by pH interaction).

permeability of the colon to ³H-mannitol in the control and the LPS-treated groups was different (137.01 vs. 29.1 nmol/cm²/h; $P < 0.05$), whereas there were no differences due to LPS at pH of 6.5 and 7.4.

Permeability of Rumen and Colon Tissues to LPS

There was translocation of LPS from the mucosal to the serosal side of the Ussing chamber in all rumen tissues treated with LPS ($P < 0.01$). The amount of LPS that went through the rumen tissue at pH 4.5, 5.5, and 6.5 was 361, 431, and 312 nmol/cm²/h for treatments vs. 0, 24, and 6.5 nmol/cm²/h for controls, respectively. Still, exposure of rumen tissues to different acidic pH

Figure 3. Translocation of LPS from *Escherichia coli* B:055 across rumen tissue obtained from steers under different pH values and in presence (■) or absence (□) of LPS from *E. coli* B:055 in the mucosal reservoir of Ussing chamber (Trt = treatment; pH = pH value of perfusate; Trt × pH = treatment by pH interaction).

did not affect translocation of LPS and there was no difference in the quantity of LPS translocated between the different pH groups (Figure 3). Furthermore, no interaction ($P > 0.05$) between pH and LPS was obtained. Evidently, LPS was translocated from the mucosal to the serosal side of the Ussing chamber at all 3 pH values when the colon tissues were exposed to LPS ($P < 0.01$; Figure 4). Thus, the amount of LPS that permeated through the colon tissues at pH 5.5, 6.5, and 7.4 was 149, 219, and 167 nmol/cm²/h in treatments vs. 0, 24, and 0 nmol/cm²/h in controls, respectively. All 3 pH values did not affect translocation of LPS through the colon tissues, and no interaction ($P > 0.05$) between pH and LPS was obtained.

Figure 2. Permeability of colon tissue obtained from steers to ³H-mannitol under different pH and in presence (■) or absence (□) of LPS from *Escherichia coli* B:055 in an Ussing chamber (Trt = treatment; pH = pH value of perfusate; Trt × pH = treatment by pH interaction).

Figure 4. Translocation of LPS from *Escherichia coli* B:055 across colon tissue obtained from steers under different pH values and in presence (■) or absence (□) of LPS from *E. coli* B:055 in the mucosal reservoir of Ussing chamber (Trt = treatment; pH = pH value of perfusate; Trt × pH = treatment by pH interaction).

DISCUSSION

The most important finding of this study was that in presence of LPS at acidic pH values of the perfusate similar with ARA (i.e., pH 4.5 and 5.5 for rumen and colon, respectively) there was an increase of more than 6-fold and 5-fold in the permeability of rumen and colon tissues, respectively, to ^3H -mannitol. Conversely, pH values similar with SARA and normal pH values of the perfusate did not affect permeability of rumen and colon mucosal tissues to ^3H -mannitol. Whereas LPS and acidic pH are known to increase the permeability of the mucosa separately (Chin et al., 2006), the increased permeability observed in our study at both acidic pH of the perfusate and presence of LPS suggests that the 2 factors combine to further enhance the permeability of mucosa to ^3H -mannitol. Tests of gastrointestinal tissue permeability to low molecular weight carbohydrates like mannitol have a considerable application in the study of gastrointestinal pathologies. Mannitol is a non-metabolizable sugar with a molecular weight of 182 Da and diameter of 0.67 nm. Normally, mannitol crosses the epithelial layers through water-filled pores and is commonly used as an intestinal permeability probe (Hollander et al., 1988). Changes in the integrity of the epithelial tissues are associated with enhanced permeability to mannitol. Therefore, increased permeability to ^3H -mannitol in our experimental conditions involving low acidic pH plus LPS suggest alterations in the integrity of the mucosal layers during ARA in cattle.

Although the mechanism(s) by which low pH and presence of LPS enhanced permeability of rumen and colon tissues to ^3H -mannitol is not clear; studies examining the effects of acidic pH on mucosal permeability reveal that acidic environments affect the permeability of mucosal epithelial layers by increasing production of NO in enterocytes (Unno et al., 1997). Also, LPS induced production of NO in various enterocytic cell lines in vitro (Dignass et al., 1995). Presence of high concentrations of NO may inhibit production of ATP and damage enterocytes (Unno et al., 1997). Our data, with rumen and colon tissues, indicated that lowering the pH in presence of LPS increased the permeability to ^3H -mannitol. The increased permeability at a pH of 4.5 for rumen and a pH of 5.5 for colon tissues, in presence of LPS, may be due to combined disrupting effects of LPS and acidic pH on the epithelial barrier functions.

Another significant finding of the present study was that translocation of LPS across the rumen and colon mucosa was not pH dependent. Lipopolysaccharide permeated through these tissues at the rates presented in Figures 3 and 4 and at concentrations used in our study (i.e., 500 $\mu\text{g/mL}$). A small amount of LPS permeated through the rumen and colon mucosal tissues in the

control chambers. Although no LPS was added in those chambers it is possible that bacteria attached to the mucosal layers contributed the small amounts of LPS measured. Results of this study confirm previous research conducted by Drewe et al. (2001) indicating that translocation of LPS through mucosal layers is not dependent on pH (pH 6.0 to 8.0). It's not clear how LPS permeates through mucosal tissues; however, Drewe et al. (2001) demonstrated that translocation of LPS can take place both by simple diffusion through transcellular pathways as well as by paracellular pathway and that translocation was not related to the presence of Na^+ ions. The latter indicates that no Na^+ -pumps (i.e., active transport) are involved in transportation of LPS.

Our finding that LPS translocated if present at high concentrations in the gut is reinforced by several other studies. For example, enteral administration of endotoxin in mice resulted in elevation of concentration of endotoxin in blood compared with control mice (Schwarzenberg and Bundy, 1994). In vitro studies with isolated gut segments demonstrated that the flux of LPS was not proportional to concentration of LPS present in the mucosal side, but rather the transport system attained a saturated state with a threshold concentration (Nolan et al., 1977). It is possible that concentration of LPS, which we used in the present study (500 $\mu\text{g/mL}$), was sufficient to disrupt the mucosal barrier functions and result in translocation of LPS to the serosal side.

Overall, results of this study suggest that under acidic pH and high concentrations of endotoxin in the rumen or colon, as reported during feeding of cattle high-grain diets, there is translocation of endotoxin and alteration of the permeability of mucosal tissues. Further research is warranted to understand the pathway that endotoxin follows after translocation into the serosal side as well as the mechanism(s) by which acidic pH and presence of LPS make rumen and colon tissues "leaky".

CONCLUSIONS

In conclusion, the permeability of rumen and colon tissues to ^3H -mannitol increased 6- and 5-fold, respectively, in presence of LPS from *E. coli* B:055 and acidic pH of pH 4.5 and 5.5, respectively. No translocation of ^3H -mannitol occurred when the pH on the mucosal side was at pH 5.5 and 6.5 for rumen tissues and pH 6.5 and 7.4 for colon tissues. Translocation of LPS occurred across the rumen and colon tissues independently of all pH values tested.

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REFERENCES

- Ametaj, B. N., B. J. Bradford, G. Bobe, R. A. Nafikov, Y. Lu, J. W. Young, and D. C. Beitz. 2005. Strong relationship between mediators of the acute phase response and fatty liver in dairy cows. *Can. J. Anim. Sci.* 85:165–175.
- Andersen, P. H., M. Hesselholt, and N. Jarlov. 1994. Endotoxin and arachidonic acid metabolites in portal, hepatic and arterial blood of cattle with acute ruminal acidosis. *Acta Vet. Scand.* 35:223–234.
- Chin, A. C., A. N. Flynn, J. P. Fedwick, and A. G. Buret. 2006. The role of caspase-3 in lipopolysaccharide-mediated disruption of intestinal epithelial tight junctions. *Can. J. Physiol. Pharmacol.* 84:1043–1050.
- Datta, A. K., and S. Basu. 1999. Chemical characterization of the lipopolysaccharides from enteropathogenic *Escherichia coli* O142 and O158. *Indian J. Biochem. Biophys.* 36:55–58.
- Dignass, A. U., D. K. Podolsky, and D. Rachmilewitz. 1995. NO₂ generation by cultured small intestinal epithelial cells. *Dig. Dis. Sci.* 40:1859–1865.
- Dirksen, V. G., H. G. Liebich, G. Brosi, H. Hagemeister, and E. Mayer. 1984. Morphology of the rumen mucosa and fatty acid absorption in cattle—Important factors for health and production. *Zentralbl. Veterinaermed.* 31:414–430.
- Drewe, J., C. Beglinger, and G. Fricker. 2001. Effect of ischemia on intestinal permeability of lipopolysaccharides. *Eur. J. Clin. Invest.* 31:138–144.
- Gozho, G. N., D. O. Krause, and J. C. Plaizier. 2007. Ruminant lipopolysaccharide concentration and inflammatory response during grain-induced subacute ruminal acidosis in dairy cows. *J. Dairy Sci.* 90:856–866.
- Grauke, L. J., I. T. Kudva, J. W. Yoon, C. W. Hunt, C. J. Williams, and C. J. Hovde. 2002. Gastrointestinal tract location of *Escherichia coli* O157:H7 in ruminants. *Appl. Environ. Microbiol.* 68:2269–2277.
- Hollander, D., D. Ricketts, and C. A. R. Boyd. 1988. Importance of “probe” molecular geometry in determining intestinal permeability. *Can. J. Gastroenterol.* 2:35–38.
- Keunen, J. E., J. C. Plaizier, L. Kyriazakis, T. F. Duffield, T. M. Widowski, M. I. Lindinger, and B. W. McBride. 2002. Effects of a subacute ruminal acidosis model on the diet selection of dairy cows. *J. Dairy Sci.* 85:3304–3313.
- Nagaraja, T. G., E. E. Bartley, L. R. Fina, and H. D. Anthony. 1978a. Relationship of rumen gram-negative bacteria and free endotoxin to lactic acidosis in cattle. *J. Anim. Sci.* 47:1329–1336.
- Nagaraja, T. G., E. E. Bartley, L. R. Fina, H. D. Anthony, and R. M. Bechtel. 1978b. Evidence of endotoxins in the rumen bacteria of cattle fed hay or grain. *J. Anim. Sci.* 47:226–234.
- Nagaraja, T. G., and G. Town. 1990. Ciliated protozoa in relation to ruminal acidosis and lactic acid metabolism. Page 187–194 in *Rumen Ecosystem: Microbial Metabolism and Regulation*. R. Oneora, H. Minato, and H. Itabashi, ed. Springer-Verlag, New York, NY.
- Nocek, J. E. 1997. Bovine acidosis: Implications on laminitis. *J. Dairy Sci.* 80:1005–1028.
- Nolan, J. P., D. K. Hare, J. J. McDevitt, and M. V. Ali. 1977. In vitro studies of intestinal endotoxin absorption. I. Kinetics of absorption in the isolated everted gut sac. *Gastroenterol.* 72:434–439.
- Salehi, P., J. Walker, K. L. Madsen, G. T. Sigurdson, B. L. Strand, B. E. Christensen, L. D. Jewell, and T. A. Churchill. 2007. Relationship between energetic stress and pro-apoptotic/cytoprotective kinase mechanisms in intestinal preservation. *Surgery* 141:795–803.
- SAS Institute Inc. 1989. SAS/STAT User's guide. SAS Institute, Cary, NC.
- Schwarzenberg, S. J., and M. Bundy. 1994. Ursodeoxycholic acid modifies gut-derived endotoxemia in neonatal rats. *Pediatr. Res.* 35:214–217.
- Silipo, A., R. Lanzetta, D. Garozzo, P. Lo Cantore, N. S. Iacobellis, A. Molinaro, M. Parrilli, and A. Evidente. 2002. Structural determination of lipid A of the lipopolysaccharide from *Pseudomonas reactans*. A pathogen of cultivated mushrooms. *Eur. J. Biochem.* 269:2498–2505.
- Unno, N., M. J. Menconi, M. Smith, D. E. Aguirre, and M. P. Fink. 1997. Hyperpermeability of intestinal monolayers is induced by NO: Effect of low extracellular pH. *Am. J. Physiol.* 272:923–934.