Investigation of the effect of black walnut extract on in vitro ion transport and structure of equine colonic mucosa

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Objective—To examine the secretory response (in the presence and absence of prostaglandin inhibition) in vitro and structural alterations of colonic mucosa in horses after intragastric administration of black walnut extract (BWE).

Animals—14 adult horses.

Procedure—Seven horses were administered BWE intragastrically and monitored for 11 hours. Tissue samples were obtained from the right ventral, left ventral, and right dorsal colons (RVC, LVC, and RDC, respectively) of the 7 BWE-treated and 7 control horses. Tissue samples were examined via light microscopy, and the extent of hemorrhage, edema, and granulocytic cellular infiltration (neutrophils and eosinophils) was graded. Colonic mucosal segments were incubated with or without flunixin meglumine (FLM) for 240 minutes; spontaneous electrical potential difference and short-circuit current (Isc) were recorded and used to calculate mucosal resistance.

Results—Colonic tissues from BWE-treated horses (with or without FLM exposure) had an overall greater Isc during the 240-minute incubation period, compared with tissues from control horses. The resistance pattern in RVC, LVC, and RDC samples (with or without FLM exposure) from BWE-treated horses was decreased overall, compared with control tissues (with or without FLM exposure). Histologically, colonic mucosal tissues from BWE-treated horses had more severe inflammation (involving primarily eosinophils), edema, and hemorrhage, compared with tissue from control horses.

Conclusions and Clinical Relevance—In horses, BWE administration appears to cause an inflammatory response in colonic mucosal epithelium that results in mucosal barrier compromise as indicated by decreased mucosal resistance with presumed concomitant electrogenic chloride secretory response, which is not associated with prostaglandin mediation. (*Am J Vet Res* 2005;66:443–449)

Elucidation of the pathophysiologic mechanisms associated with development of colic and laminitis is of interest to many researchers, and in a recent large-scale survey[1] of equine practitioners, these were the most important disease categories that respondents felt needed further investigation. As global research efforts continue to help explain specific theories on the pathogenesis of these causes of illness and death, the interconnection between the 2 diseases is well accepted and justifies further examination.

There is a very strong correlation between colic, endotoxemia, and the onset of laminitis in clinical equine practice.[2,3] The cascade of events that results in the development of colic and the associated episode of acute laminitis that often develops subsequently in horses has been hypothesized to involve endotoxemia.[4] However, administration of endotoxin does not consistently cause laminitis in horses.[5] In studies by Krueger et al[6] and Weiss et al,[7] substantial intestinal mucosal damage was detected in horses that received experimental overload of carbohydrate to induce laminitis, which further supports the link between colic and laminitis. In horses, a relationship between colic and laminitis in association with exposure to black walnut shavings has also been identified but the exact mechanism has not been elucidated. Although the most serious clinical sign of black walnut toxicosis in horses is laminitis, affected horses may also have signs of lethargy, anorexia, respiratory distress, and colic.[8-10] In horses, the gastrointestinal tract abnormalities associated with black walnut ingestion have not been well characterized. Although True and Lowe[8] failed to consistently induce laminitis in horses and ponies via intragastric administration of juglone (a proposed toxic compound of black walnut), those investigators did observe increased borborygmi or ileus and mild signs of colic in 4 of 10 treated equids. In another study[9] in which 10 horses were exposed to black walnut shavings, 2 of 10 horses had signs of abdominal discomfort.

The induction of laminitis via intragastric administration of **black walnut extract (BWE)** is a well-recognized research method for studying this catastrophic disease in horses.[10,11] Compared with the carbohydrate overload method of laminitis induction, the BWE method is more reliable and clinical signs of laminitis

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are detected within the first 12 hours after treatment in > 90% of experimental animals.[10-13] In endeavors to gain more insight into the connection between colic and laminitis in horses, the induction of laminitis via intragastric administration of BWE provides the opportunity to study the integrated nature of cellular elements in equine colonic epithelium and understand the local immune response as it relates to mucosal secretion and absorption. The effects of a commonly used prostaglandin inhibitor, flunixin meglumine, on mucosal integrity in horses with colic have also been investigated.[14] We hypothesized that BWE administered via nasogastric intubation to horses would perturb the equine colonic mucosa, causing structural damage and a change in electrogenic chloride secretion. In addition, we hypothesized that addition of a prostaglandin inhibitor to colonic tissue samples would ameliorate the secretory response in vitro. The purpose of the study reported here was to examine the secretory response (in the presence and absence of prostaglandin inhibition) in vitro and structural alterations of colonic mucosa in horses after intragastric administration of BWE.

Materials and Methods

Animals and tissue preparation—This study was approved by the Louisiana State University Institutional Animal Care & Use Committee in compliance with the Animal Welfare Act, US Public Health Service Policy on the Humane Care and Use of Laboratory Animals. Seven light-breed horses (1 mare and 6 geldings) that were 5 to 17 years of age and weighed 411 to 591 kg were included in the study. Each horse was determined to be free of laminitis and gastrointestinal tract disease on the basis of history, findings of thorough physical and lameness examinations, and evaluation of lateral radiographs of both front feet.[a] Horses received standard anthelmintic and health maintenance treatments and were housed in stalls and fed grass hay. Prior to administration of BWE, heart and respiratory rates were assessed and a blood sample was obtained via jugular venipuncture from each horse allocated to the treatment group for determination of PCV; WBC, neutrophil, and platelet concentrations; and plasma fibrinogen and total protein concentrations. A baseline lameness examination was performed, and an Obel grade[15] was assigned to each horse.

Horses were administered BWE (2 g/kg) via nasogastric tube.[11] During an 11-hour period, the horses were monitored continuously for signs of depression and abdominal discomfort and changes in appetite and hourly for capillary refill time and gastrointestinal tract motility (assessed via auscultation of borborygmal sounds). A blood sample was obtained via jugular venipuncture hourly from each horse allocated to the treatment group for determination of PCV; WBC, neutrophil, and platelet concentrations; and plasma fibrinogen and total protein concentrations. Approximately 11 hours after BWE administration, anesthesia was induced via IV administration of xylazine hydrochloride (0.50 to 1.0 mg/kg), sodium thiopental (10 mg/kg), and sodium pentobarbital (7.5 mg/kg); anesthesia was maintained with sodium pentobarbital (5 to 15 mg/kg/h, IV). Horses were positioned in right lateral recumbency and ventilated with positive pressure and 100% oxygen. Horses were anesthetized for 40 to 60 minutes while colonic samples were obtained via left paramedian celiotomy. One full-thickness tissue segment (10 × 10 cm) was obtained from each of 3 sites: the antimesenteric side of the **right ventral colon (RVC)**, **left ventral colon (LVC)**, and **right dorsal colon (RDC)**. Without recovering from anesthesia, horses were euthanized by use of an overdose of sodium pentobarbital. For comparison, samples were obtained from the same segments of the large colon of 7 healthy control horses immediately after euthanasia (by use of an overdose of sodium pentobarbital); these horses were euthanized for reasons other than laminitis and gastrointestinal tract disease. Colonic tissues were rinsed with ice-cold saline (0.9% NaCl) solution, then immediately transferred to dissecting pans and bathed in ice-cold buffered 0.9% Ringer's solution (pH, 7.4). The Ringer's solution contained Na, 140 mmol/L; K, 5.2 mmol/L; Ca, 2 mmol/L; Mg^{2+}, 1.2 mmol/L; Cl^-, 119.8 mmol/L; HCO_3^-, 25 mmol/L; $H_2PO_4^-$, 0.4 mmol/L; HPO_4^{2-}, 2.4 mmol/L; and glucose, 10 mmol/L.

Preparation of extract—The BWE was prepared as previously described.[12,13] Briefly, shavings were made from the heartwood of a black walnut tree that had been cut in the fall of the year and were stored at –20°C until used. For treatment of each horse, 2 g of black walnut shavings/kg of body weight were combined with 8 L of distilled water and mixed in a shaker bath[b] for 14 hours at room temperature (20° to 22°C). The mixture was filtered and refrigerated, and the fluid was administered within 24 hours of preparation.

Ion transport experiments—Colonic tissue samples were submerged in Ringer's solution that did or did not contain a prostaglandin inhibitor, and the serosal and muscular layers were removed via sharp dissection. To prevent prostaglandin elaboration during the stripping procedure, flunixin meglumine[c] (2.7×10^{-5} mol/L) was added to tissues that would be treated with the same prostaglandin inhibitor in the incubation solution during the transport studies. Mucosal samples from the RVC, LVC, or RDC were mounted in Ussing chambers[d] and bathed in warm (38°C) buffered Ringer's solution gassed with a continuous flow of 95% oxygen and 5% carbon dioxide. Samples were placed in chambers in triplicate according to region of intestine examined and treatment (ie, with or without exposure to flunixin meglumine). Only 12 chambers were available for use; therefore, only 2 regions of the intestine could be evaluated from each horse. Samples from the RVC and LVC of 3 BWE-treated and 3 control horses were evaluated, samples from the LVC and RDC of 2 BWE-treated and 2 control horses were evaluated, and samples from the RVC and RDC of 2 BWE-treated and 2 control horses were evaluated. Bathing solutions were connected to calomel electrodes[e] via Ringer agar bridges. The tissues were short circuited continuously via Ag-AgCl electrodes connected to the chambers via agar bridges. The tissues were allowed to equilibrate for 30 minutes prior to recording. The **short-circuit current (Isc)** and open-circuit potential difference were recorded manually every 15 minutes for 210 to 240 minutes. By application of Ohm's law (ie, voltage equals current multiplied by resistance), tissue resistance was calculated by use of potential difference and Isc values.

Histologic evaluations—After removal, duplicate tissue samples for histologic evaluation were placed in neutral-buffered 10% formalin. Samples were embedded in paraffin, cut in 5-µm sections, and stained with H&E for examination. Sections of colonic mucosa from each segment of the colon were examined via light microscopy by investigators (RSM and RMM) who were unaware of the sample treatment. Tissue samples were subjectively graded with respect to edema, focal hemorrhage, and granulocytic cell infiltration by use of a scoring system of 0 to 3. A score of zero indicated no hemorrhage or edema observed. A score of 1 indicated slight to mild hemorrhage or edema after close inspection of the tissue section. A score of 2 was indicative of moderate hemorrhage or edema (ie, extensive changes but no distortion of normal tissue architecture). A score of 3 was indicative of marked to severe hemorrhage or edema (with distor-

tion of normal tissue architecture). The extent of granulocytic cell infiltration associated with inflammation was subjectively assessed, and each section was assigned a grade of 0 to 3 (0 = normal tissue with no or few granulocytes [eosinophils and neutrophils]; 1 = minimal inflammation with minimal to low numbers of scattered granulocytes; 2 = moderate inflammation with noticeable numbers of granulocytes with foci in the mucosa; 3 = marked inflammation with many granulocytes with foci in submucosa and vessels).[16,17] The 2 investigators' scores were averaged for each sample.

Statistical analyses—In vitro electrical data were analyzed by use of a 2-way ANOVA[f] on repeated measures and post hoc comparison. Differences in histologic grades between the BWE-treated and control groups were compared by use of a Mann-Whitney test. A value of $P < 0.05$ was considered significant for all tests.

Results

Clinical findings—All horses treated with BWE developed signs of mild to moderate abdominal discomfort during the 10- to 11-hour period after administration of the extract. Other physical changes in the treated horses after BWE administration included signs of depression, increased respiratory rate, decreased borborygmal sounds (ie, absent sounds in > 2 abdominal quadrants), decreased appetite, and prolonged capillary refill time (**Table 1**). Signs of foot pain (Obel grade 1) were generally detected at 10 to 11 hours after administration of the extract. Detection of Obel grade 1 foot pain at 10 to 11 hours after BWE administration corresponded to significant ($P < 0.05$) decreases in peripheral WBC and neutrophil counts (mean ± SEM WBC count, 5.22 ± 1.29 × 10^3 WBCs/µL; mean neutrophil count, 2.54 ± 1.90 × 10^3 cells/µL) at 3 hours after BWE administration and subsequent rebound increases ($P < 0.05$) in WBC and neutrophil counts at 10 to 11 hours, compared with baseline values (**Table 2**).

Tissue resistance and ion transport experiments—Mean time-0 values of Isc (obtained after an equilibration period of 20 to 30 minutes) were significantly greater for mucosal segments from all areas of intestine in horses that had received intragastric administration of BWE, compared with tissues from healthy control horses (**Figure 1**). The addition of flunixin meglumine (2.7×10^{-5} mol/L) to either BWE-treated tissues or control tissues did not affect the values of Isc at time 0 for each respective mucosal segment (RVC, LVC, or RDC).

Compared with tissues from control horses, Isc was significantly greater in mucosal tissue from BWE-treated horses at time 0 through 150 minutes for RVC samples, time 0 through 180 minutes for LVC samples, and time 0 through 240 minutes for RDC samples. Right dorsal colonic tissue (with or without flunixin meglumine exposure) from BWE-treated horses had an overall significant increase in Isc during the 210- to 240-minute incubation period, compared with that of control tissues. The exposure of BWE-treated RDC mucosa to flunixin meglumine resulted in no difference in Isc during the entire incubation period. Short-circuit current in RVC tissue (with or without flunixin meglumine exposure) from BWE-treated horses remained constant and was significantly greater than that of control tissues throughout the incubation period until the 150-minute time point. Subsequently, Isc

Table 1—Number of horses (n = 7) with certain physical examination findings during an 11-hour period after intragastric administration of black walnut extract (BWE). Among the 7 horses, the mean interval after BWE treatment to development of Obel grade 1 lameness was 10 hours.

	Time after BWE administration (h)		
Physical examination variable	**1–3**	**4–6**	**7–11**
Signs of depression	2	2	3
Signs of abdominal discomfort (kicking at abdomen, looking at side, stretching)	6	2	2
Decreased appetite	1	1	1
Prolonged capillary refill time (> 2 s)	2	1	2
Decreased gastrointestinal tract motility (decreased sounds in > 2 quadrants)	3	0	1

Table 2—Mean ± SEM hematologic and physical examination variables in 7 horses before (baseline) and at 10 hours after intragastric administration of BWE (mean interval after BWE treatment to development of Obel grade 1 lameness was 10 hours).

Variable	**Baseline**	**10 hours after administration of BWE**
PCV (%)	32.5 ± 2.6	34.3 ± 3.5
Total plasma protein concentration (g/dL)	6.98 ± 0.43	6.87 ± 0.54
WBC concentration* (× 10^3 cells/µL)	8.458 ± 1.262	11.729 ± 3.495
Neutrophil concentration† (× 10^3 cells/µL)	4.969 ± 3.420	8.771 ± 5.125
Platelet concentration (× 10^3 platelets/µL)	147.233 ± 33.196	141.571 ± 37.872
Fibrinogen concentration (mg/dL)	245.2 ± 71.1	242.9 ± 97.6
Temperature (°C)	38.0 ± 0.18	38.8 ± 0.76
Heart rate (beats/min)	36.9 ± 2.57	41.7 ± 10.61
Respiratory rate (breaths/min)	11.8 ± 3.2	19.1 ± 11.0
Obel grade of lameness	0 ± 0	0.71 ± 0.76

*At 3 hours after BWE treatment, value was decreased by > 40% (5.23 ± 1.29 × 10^3 cells/µL), compared with baseline value. †At 3 hours after BWE treatment, value was decreased by > 40% (2.54 ± 1.90 × 10^3 cells/µL), compared with baseline value.

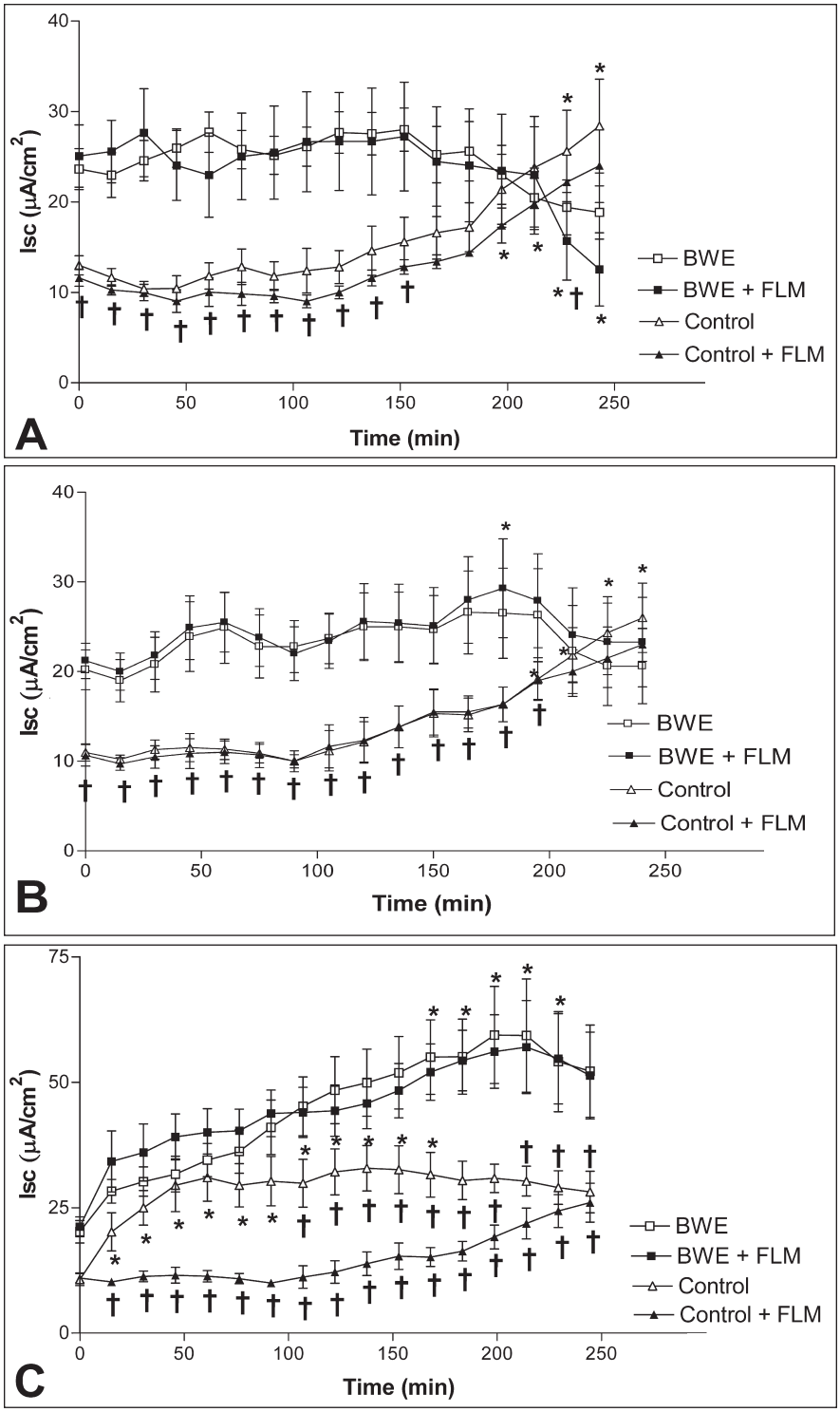

Figure 1—Short-circuit current (Isc) in samples of right ventral colon (RVC; A), left ventral colon (LVC; B), and right dorsal colon (RDC; C) obtained from horses 11 hours after intragastric administration of black walnut extract (BWE) and healthy control horses. Tissues from treated and control horses were incubated for 210 to 240 minutes with or without flunixin meglumine (FLM; 2.7 X 10^{-5} mol/L). Because of logistic reasons, samples of RVC from 5 control and 5 BWE-treated horses, samples of LVC from 5 control and 5 BWE-treated horses, and samples of RDC from 4 control and 4 BWE-treated horses were evaluated. *Value significantly ($P < 0.05$) different at that time point, compared with the value for that tissue group at the beginning of the incubation period. †Value significantly ($P < 0.05$) different from comparison group (ie, tissue from BWE-treated horses with FLM vs tissue from control horses with FLM or tissue from BWE-treated horses without FLM vs tissue from control horses without FLM) at the same incubation time. Data are expressed as least-squares mean ± SEM.

values for RVC tissue from BWE-treated horses decreased significantly, compared with control tissue sample readings. A similar pattern was observed in the LVC (with or without flunixin meglumine exposure) from BWE-treated horses from time 0 to 165 minutes (Figure 1). The Isc value for the RVC and LVC mucosal samples from control horses increased steadily beginning at 105 and 120 minutes of incubation, respectively; the increase continued until tissues were removed from the incubation solution after 210 to 240 minutes. In RDC tissues from control horses, the addition of flunixin meglumine resulted in an overall significant decrease in Isc, compared with the paired tissues incubated without exposure to flunixin meglumine.

Right ventral colonic tissues from BWE-treated horses had an overall reduction in resistance (with or without flunixin meglumine exposure), compared with that of control tissues (with or without flunixin meglumine exposure), during the 240-minute period (**Figure 2**). A similar pattern of tissue resistance was observed in LVC and RDC samples (data not shown). For all large intestinal sites examined, tissue resistance of colonic samples peaked at 60 to 90 minutes of incubation and remained steady until values gradually decreased after 3 hours of incubation.

Pathologic tissue changes—Compared with tissues from control horses, there were significant increases in inflammation, edema, and hemorrhage in samples of colonic mucosa from BWE-treated horses (**Table 3**). Histologic grades in all mucosal samples (RVC, LVC, and RDC) from BWE-treated horses were consistently greater than grades in the samples from control horses. Samples from the RDC of control horses had a significantly higher grade of granulocytic cell infiltration (predominantly eosinophils), compared with samples of RVC and LVC from control animals.

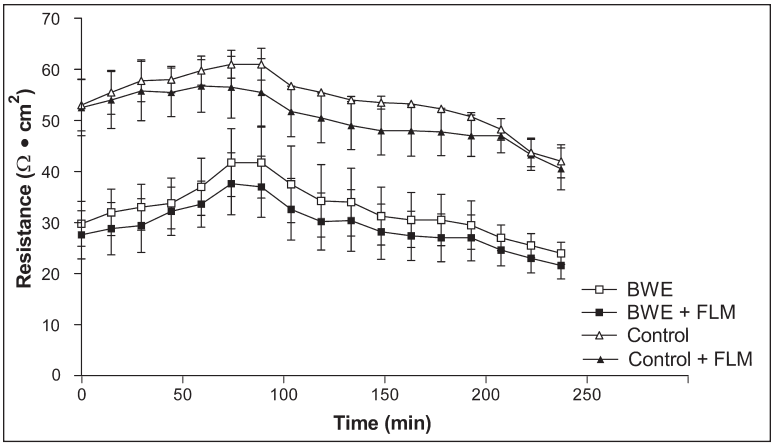

Figure 2—Tissue resistance for RVC samples obtained from 5 horses 11 hours after intragastric administration of BWE and 5 healthy control horses. Resistance was calculated on the basis of potential difference and Isc values by application of Ohm's law. During the 240-minute period, there was a pattern of decreased resistance in tissues (with or without FLM exposure) from BWE-treated horses, compared with tissues (with or without FLM exposure) from control horses. Data are expressed as least-squares mean ± SEM.

Table 3—Mean ± SEM histologic grade (0 to 3) of the extent of edema, hemorrhage, and inflammation and cellular infiltration (eosinophils and neutrophils) of the mucosal epithelium and submucosa in samples of right ventral colon (RVC), left ventral colon (LVC), and right dorsal colon (RDC) obtained from 7 horses after intragastric administration of BWE and 7 control horses. Cellular infiltration was subjectively assessed as normal (grade 0, no to few granulocytes [eosinophils and neutrophils]), minimal (grade 1, minimal to low numbers of scattered granulocytes), moderate (grade 2, noticeable number of granulocytes with foci in the mucosa), or marked (grade 3, many granulocytes with foci in submucosa and vessels).

Region of intestine Treatment	Grade of histologic variable Edema	 Hemorrhage	 Inflammation and cellular infiltration
RVC			
Control	0.330 ± 0.167	0.667 ± 0.236	1.000 ± 0.288
BWE	1.330 ± 0.289*	2.111 ± 0.200*	2.780 ± 0.147*
LVC			
Control	0.400 ± 0.163	0.900 ± 0.233	0.900 ± 0.233
BWE	1.400 ± 0.163*	2.000 ± 0.258*	2.700 ± 0.153*
RDC			
Control	0.250 ± 0.163	0.875 ± 0.295	1.000 ± 0.289
BWE	1.375 ± 0.183*	2.250 ± 0.250*	2.375 ± 0.324*

*Value significantly different ($P < 0.05$) from that of control tissues from the same intestinal region.

Discussion

In horses, experimental induction of laminitis via intragastric administration of BWE provides an opportunity to investigate the potential link between gastrointestinal tract disease and laminitis. Several researchers[3,8,9] have reported signs of colic associated with natural or experimental ingestion of black walnut in horses. Gastrointestinal tract disease was the most common problem in 19 of 35 (54%) horses exposed to black walnut shavings, which developed just prior to the onset of acute laminitis in 1 study.[8] In horses with laminitis induced via carbohydrate overload, it has been suggested that changes in the microbial population and lactic acid production in the cecum, combined with endotoxin absorption across the damaged cecal mucosal barrier, are involved in the early onset of acute laminitis.[4,18] Results of other studies[6,7] also suggest that intestinal mucosal barrier damage is present during the acute onset of laminitis in equids. Cecal ultrastructural changes, including epithelial surface sloughing, absence of microvilli, pyknotic nuclei, and the presence of vacuoles, were observed from 24 to 72 hours after carbohydrate administration; these changes were coincident with onset of laminitis in the study horses.[6]

In a study of ponies by Weiss et al,[19] changes in Isc, conductance, and large molecule permeability in RVC tissue in response to treatment with cecal contents incubated with starch to mimic the carbohydrate overload model of laminitis were evaluated in vitro. The results of that study suggested that increased colonic mucosal permeability can develop in vitro. However, limited scientific information is available describing pathophysiologic events occurring in the intestine of equids at the time of acute onset of laminitis. In the present in vitro study, we evaluated colonic mucosal Isc in tissue samples obtained from 7 healthy control horses and 7 horses approximately 11 hours after administration of BWE; in the treated horses, the col-

lection of tissue samples coincided with development of Obel grade 1 laminitis.[15,20] In addition, we examined intestinal tissues histologically and evaluated structural tissue changes in treated and control horses. Our data indicated that BWE administration resulted in an inflammatory response in the colonic mucosal epithelium; the subsequent inflammatory cascade may contribute to an overall decrease in mucosal tissue resistance and an electrogenic chloride secretory response in equine intestinal tissue.

Three areas of the large intestine (RVC, LVC, and RDC) were evaluated to determine whether BWE treatment resulted in regional differences in mucosal tissue structure (as described previously[17] in cases of naturally occurring and experimentally induced colitis), Isc, and resistance. Short-circuit current is the charge flow per time when the tissue is short circuited with the flow of ions per time and usually indicates the secretory capacity of the tissue.[21] However, because unidirectional ion flux studies were not performed in our study, we were not able to distinguish between secretory and malabsorptive processes. Only 12 Ussing chambers were available at a time in the present study; therefore, the number of samples that could be evaluated at 1 time was limited (ie, samples of RVC from 5 control and 5 BWE-treated horses, samples of LVC from 5 control and 5 BWE-treated horses, and samples of RDC from 4 control and 4 BWE-treated horses). The 3 specific colonic regions evaluated in our study were selected because of the accessibility of those tissues via a ventral left paramedian celiotomy, previous evidence[17,22] of structural inflammatory changes in equine colonic tissue as a result of colic episodes, and previously reported techniques[23-25] for evaluation of Isc in tissues from these regions.

Samples were incubated either in the presence or absence of the prostaglandin inhibitor flunixin meglumine because this is an agent that is commonly administered to ameliorate prostaglandin-mediated abdominal pain in horses with colic. In BWE-treated tissues, the rate of electrogenic chloride secretion was much higher in both RVC and LVC samples (the principal site of fermentation), compared with control horse mucosal tissues at these sites, and was not affected by the addition of flunixin meglumine in vitro. The RDC segments from BWE-treated horses responded with a marked and steady increase in Isc throughout the 240-minute evaluation period with and without flunixin meglumine exposure, which suggested there was nonprostanoid pathway involvement in this response. However, among RDC tissue segments from control horses, those without flunixin meglumine exposure had an increased Isc from 30 minutes of incubation through 210 minutes, compared with those bathed with flunixin meglumine during the same period. The flunixin meglumine-ameliorated Isc response in RDC tissue from control horses may be a result of a higher level of prostaglandin reactivity in this area of intestine, compared with that in other areas.[23] Excessive prostanoid synthesis in RDC tissue has been proposed[17,23] and may suggest a possible theory for the response detected in those tissues in the present study. The difference in mucosal electrical responses between RDC tissues from BWE-treated horses and control horses detected in our study is interesting; it could possibly indicate that this area is unique and under the influence of other important pathways that cause epithelial dysfunction such as upregulation of proinflammatory mediators, cytokines, or cell to cell crosstalk between epithelial barrier components. Additional more refined studies would be necessary to elucidate these potential epithelial barrier regulatory pathways.

Resistance, as calculated by Ohm's law, is generally thought to be a reflection of epithelial barrier permeability and is associated with the integrity of intercellular tight junctions located at the apical side of epithelial cells. In the study of this report, resistance in all 3 areas of the large intestine examined was consistently greater in tissues from control horses throughout the 240-minute incubation period, compared with that of tissues from BWE-treated horses. The addition of flunixin meglumine did not significantly alter resistance in colonic tissues from either control or BWE-treated horses. This suggested that, in horses, tight junction integrity is altered in response to BWE ingestion and that prostaglandin-mediated pathways are not likely to be involved. It is possible that downregulation of specific epithelial tight junction proteins, such as occludin, resulted in the permeability changes observed in our study.[24] Further understanding of the specific mechanisms underlying regulation of intestinal epithelial tight junctions in this model of laminitis would be necessary to determine the link between black walnut toxicosis and the chain of events leading to the predictable onset of laminitis in horses.

The increase in Isc of RDC tissue from BWE-treated horses despite detection of a steady tissue resistance pattern could imply that a temporal chemokine effect may exist within the epithelial barrier that results in upregulation of immune cells and cytokines via subsequent calcium-mediated secretion, but it is unlikely to be related to tight junction integrity. The changes detected in the Isc values of the RDC samples (but not in RVC or LVC samples) from BWE-treated horses suggest the possibility of an increased susceptibility of this anatomic site to the effects of BWE ingestion, compared with the susceptibility of the other 2 areas of intestine examined under these conditions.

Histologic evaluation of colonic tissue from horses administered BWE revealed consistently higher grades of inflammation severity in all intestinal segments, compared with tissues from control horses. The severity of pathologic changes was denoted by marked granulocytic infiltration (predominantly eosinophils) and hemorrhage. Eosinophils reside in intestinal mucosa in most species under normal conditions, but their exact role remains unclear.[26] Intestinal tissue eosinophilia is present in allergen-related and parasitic disease[27-29]; the involvement of eosinophils in the immune response in those conditions is considered important and includes inflammatory mediator upregulation, particularly the Th-2 response.[30] Cytokines, especially interleukin-4 and -13, are produced by non-T cells and are responsible for the increases in numbers of eosinophils and mast cells and IgE concentration.[30] Interleukin-4 plays an important role in the chloride secretory response of intestinal tissues

and is thought to be a major component of mechanisms by which various foreign substances are purged from the gastrointestinal tract.[31] More specifically, interleukin-4 causes intestinal epithelial secretion that stimulates mast cell degranulation.[32] Through various other pathways, such as enteric neuronal stimulation or via eosinophil activation and release of specific secretagogues, there is direct stimulation of intestinal epithelial secretion with a concomitant upregulation of interleukin-13.[31]

The fact that tissues from BWE-treated horses in our study were markedly infiltrated with eosinophils is noteworthy and warrants further investigation. The possibility of a local chemical toxic effect against the mucosal barrier that correlates with a dampened resistance pattern in tissues from BWE-treated horses, compared with that of tissues from control horses, cannot be ruled out and would also require further investigation.

Results of the present study indicated that BWE administration in horses results in a predominantly eosinophilic inflammatory response in colonic mucosal epithelium, which causes mucosal barrier compromise (as demonstrated by decreased mucosal resistance). In addition, these data suggest that BWE administration in horses evokes an electrogenic chloride secretory response of the epithelial barrier, which is not associated with prostaglandin mediation in the large colon but may be, in part, a consequence of tissue eosinophilic activation and pronounced inflammatory cascade. These responses appear to suggest that BWE-induced laminitis in horses may be associated with decreased intestinal mucosal integrity and concomitant local intestinal mucosal inflammation.

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