Effect of Glutamine or Glycine Containing Oral Electrolyte Solutions on Mucosal Morphology, Clinical and Biochemical Findings, in Calves with Viral Induced Diarrhea

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ABSTRACT

Twenty-one diarrheic calves were randomly assigned to 1 of 3 oral electrolyte treatments. The treatments were either a conventional oral electrolyte containing glycine (40 mmol/L) as the amino acid, an oral electrolyte in which glutamine (40 mmol/L) replaced glycine or an electrolyte in which high concentrations of glutamine (400 mmol/L) replaced glycine. The calves were monitored while on trial and at the end of the treatment they were euthanized and a necropsy was immediately performed. Calves fed the high glutamine electrolyte had more treatment failures (2/7 versus 0/7 for each of the other 2 treatments). There was a significant effect of type of electrolyte on fecal consistency. Calves fed the glycine containing electrolyte had the most solid feces. Duodenal villus height was significantly affected by the type of electrolyte: values (mean ± 1 SEM) were 0.61 ± 0.09, 0.46 ± 0.05, and 0.59 ± 0.07 mm for high glutamine, low glutamine and glycine electrolytes respectively. There was no significant difference in small intestinal surface area between groups. High glutamine treated calves had the greatest capacity to absorb xylose from the small intestine but this difference was not statistically significant. Overall, this trial does not suggest that substituting glutamine for glycine in oral electrolyte solutions improves treatment of diarrheic calves or speeds mucosal healing.

RÉSUMÉ

Des veaux diarrhéiques (*n* = 21) furent traités per os à l'aide d'une solution conventionnelle d'électrolytes contenant soit 40 mmol/L de glycine, 40 mmol/L de glutamine ou 400 mmol/L de glutamine. Des suivis clinique et pathologique ont été réalisés chez tous les veaux. Les veaux recevant les hauts niveaux de glutamine ont eu un taux d'échecs au traitement supérieur (2/7 vs 0/7) par rapport aux deux autres solutions. Le type d'électrolytes a eu un effet significatif sur la consistance des fèces; la glycine augmentant la matière solide. La longueur des villosités au niveau du duodénum était significativement influncée par le type d'électrolytes : étant respectivement de 0,61 ± 0,09, 0,46 ± 0,05 et 0,59 ± 0,07 mm pour les haute et basse concentrations en glutamine et la glycine. Par contre aucun effet n'a été décelé au niveau de la surface du petit intestin. Quoique la différence était non significative, les veaux recevant les hauts niveaux de glutamine avaient une plus grande capacité à absorber le xylose. Dans l'ensemble, cette étude démontre que le fait de substituer la glycine par la glutamine n'améliore pas la vitesse de guérison de veaux diarrhéiques.

(Traduit par docteur Pascal Dubreuil)

INTRODUCTION

Diarrhea is a major problem in calves (1,2) and children (3,4). Advances in our understanding of electrolyte losses and their replenishment through oral and intravenous routes have greatly improved survival. The main aim of oral electrolyte therapy has been to maintain the patient by replacing fluid and electrolyte losses. This sustains the patient while the intestinal mucosa regenerates. At present, there are no methods to speed this regenerative process. In previous studies we found poor xylose and lactose absorption in many diarrheic calves (5), likely resulting from mucosal damage. This limits the absorption of oral electrolytes, reduces growth rates, and if severe may cause chronic diarrhea and death. Advances in the understanding of intestinal physiology suggest that it may be possible to reverse mucosal atrophy caused by enteritis. It has been proposed that glutamine, which previously was thought to be non-essential, is the limiting amino acid for mucosal regeneration (6,7,8). A number of studies show that the mucosa preferentially utilizes glutamine (9,10,11,12) and it has been suggested that glutamine supplementation could speed intestinal recovery in a variety of disease states (6,7,8). One model that has been used to study possible benefits of glutamine is mucosal atrophy in the rat receiving no enteral nutrition. Supplemental glutamine, fed either parenterally or orally, reduces small intestinal villous atrophy in some (13,14) of the studies that used this model. Benefits from glutamine supplementation have also been documented in some models of mucosal recovery following radiation injury (15,16) or compensatory hypertrophy following massive intestinal resection (17). Glutamine also stimulates sodium transport across mucosal cells in vitro, both in normal and infected enterocytes (18–21).

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As yet there have been no in vivo studies of the potential benefits of glutamine therapy in diarrhea. The objective of the present study was to determine if addition of glutamine to oral electrolyte solutions would speed mucosal regeneration, reduce weight loss, and decrease the period of diarrhea. Two concentrations of glutamine were used. The low concentration is similar to that used for glycine in some oral electrolyte solutions designed primarily for rehydration. The high concentration is similar to that used for glucose in 'high-energy' oral electrolyte solutions designed to provide both rehydration and nutritional support. The high concentration is also similar to that shown to speed intestinal regeneration in some rat models (15).

MATERIALS AND METHODS

CALVES AND TREATMENT ALLOCATION

Holstein calves were purchased from local dairies and entered the experiment at less than 1 wk of age. Calves were managed according to the *Guide to the Care and Use of Experimental Animals*. They were housed in individual crates and given free access to water. They were infected with 10 mL of pooled feces from a diarrheic calf. The fecal sample was positive for rotavirus and coronavirus and negative for cryptosporidia, enterotoxigenic *E. coli* and *Salmonella* species. Calves were maintained on 2 L milk twice daily until they became diarrheic. At this time they were blocked into groups of 3 and were randomly assigned to 1 of 3 electrolyte solutions identical in all respects except amino acid content. One solution contained 40 mmol/L glycine, the other 40 mmol/L glutamine (low glutamine), and the last 400 mmol/L glutamine (high glutamine, Table I). Calves were fed 2 L of their respective treatment twice daily via nipple bottle. No milk was fed during the treatment period. Calves too weak to suckle received their feedings via stomach tube and the total intake of electrolyte solution intake by each calf was 4 L daily. The 1st day of diarrhea was classified as day 1 of treatment. Twenty-one diarrheic calves were randomly assigned to treatment as described above. An additional diarrheic calf was added to the high glutamine group as a replacement for a calf that was euthanized early in the trial phase.

TABLE I. Composition (mmol/L) of oral electrolyte solutions

Item	Glycine	Low glutamine	High glutamine
Na^+	120	120	120
K^+	20	20	20
H^+	12.8	12.8	12.8
HPO_4^{--}	15	15	15
$Citrate^{---}$	7.8	7.8	7.8
$Acetate^-$	80	80	80
D-Glucose	120	120	120
Glycine	40	0	0
Glutamine	0	40	400
Osmolality (Calculated)	415.6	415.6	775.6

A 2nd group of 6 calves was purchased from the same sources and maintained on 2 L of milk twice daily for 5 d as controls. These calves were not infected, were maintained in a separate room and at different times from infected calves, and were euthanized on day 6.

ASSESSMENT

Clinical assessment of hydration status, mental depression and fecal consistency was performed daily and scored using systems previously developed (22,23,24). The depression scoring system ranges from 0 in fully alert calves to 8 in maximally depressed, recumbent, comatose, calves. The fecal scoring system ranged from 0 for formed feces to 3 for watery diarrhea. Three mL of heparinized blood was collected via the jugular vein immediately pre-infection and on days 1, 3, and 5 of diarrhea. Packed cell volume (PCV) was measured after centrifugation for 5 min in a microhematocrit centrifuge (IEC MB Centrifuge, Damon, Needham Hts, Massachusetts, USA), plasma total protein (TP) was measured by refractometry. Acid-base status was determined using an ABL 330 analyzer (Radiometer, Copenhagen). Calves were weighed before feeding on the morning following purchase and every other day until they developed diarrhea. They were weighed on day 1 of the diarrheic period and then every other day until euthanasia. Prior to day 4, a jugular catheter (Angiocath, Becton-Dickinson, Sandy, Utah, USA) was placed and an extension set (Venisystems, Abbott Ireland Ltd) attached. On day 4, the morning treatments were replaced by a 1% xylose (Sigma Chemical Co, St Louis, Missouri, USA) solution. Six mL of blood was then collected into serum tubes (Terumo Medical Corp, Elkton, Maryland, USA) at −15 min, 0 h, and every hour thereafter for 7 h. The blood was then centrifuged and the serum stored at −20°C until analyzed for xylose content (25). On day 5, calves were euthanized with pentobarbital (Euthanyl Forte, MTC Pharmaceuticals, Cambridge, Ontario). After 3 calves from each electrolyte treatment had undergone treatment for 5 d, a preliminary analysis indicated that there were no treatment differences. Accordingly the remaining 4 calves from each electrolyte group underwent treatment for a total of 8 d to increase the chances of seeing a treatment effect. Xylose absorption testing was performed on day 7. Calves were euthanized on day 8.

Necropsy was performed immediately after euthanasia. The distance between the crown and rump was measured. Two 5 cm lengths each of duodenum, jejunum, ileum, proximal colon, and distal colon were removed, mounted on wood, and fixed in Bouin's solution for 24 h. Jejunum, the 1st tissue removed, was placed in fixative within 2 min of death. Tissues were processed routinely, 5 μm sections were cut and stained with hematoxylin/eosin. Histologic sections were then analyzed for villus height and crypt depth by use of an Image-1 computerized video microscopy system (Universal Imaging Corp, West Chester, Pennsylvania, USA). The entire intestine was removed and the lengths of the small and large intestine were measured. The intestines were weighed both with their contents and after opening and drainage.

CALCULATION OF SMALL INTESTINAL SURFACE AREA

Areas were calculated for each segment of the small intestine using the formula:

Surface area, m^2 =

Length × Width × Villi/mm^2 × Villus area/10 000

Table II. Selected measurements in noninfected and infected diarrheic calves

Item	Noninfected (control)	Infected (all diarrheic calves)
Number of observations	6	20
Fecal score, day1	0 ± 0	2.2 ± 0.1
Fecal score, day 5	0 ± 0	2.2 ± 0.2
Rectal temperature, °C day 1	39.0 ± 0.08	39.6 ± 0.08
Rectal temperature, °C day 5	38.9 ± 0.11	39.1 ± 0.07
Change in PCV, L/L day 5	−0.018 ± 0.024	0.024 ± 0.007
Blood pH, day 5	7.375 ± 0.009	7.403 ± 0.010
Weight change, kg, day 5	1.48 ± 0.3	−1.4 ± 0.3
Area under xylose absorption curve, mmol/h	4.3 ± 0.4	2.2 ± 0.2
Jejunal surface area, m^2	9.1 ± 1.1	5.7 ± 0.3

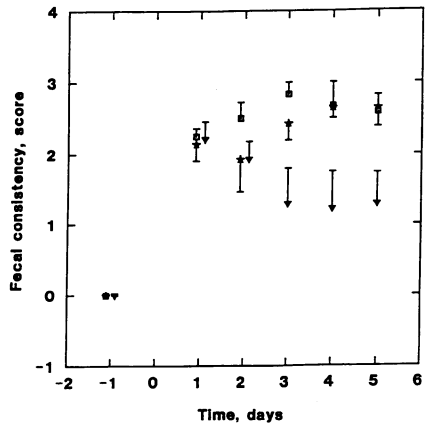

Figure 1. Severity of diarrhea in calves receiving different oral electrolyte solutions, ▽ glycine 40 mmol/L, ★ glutamine 40 mmol/L, □ glutamine 400 mmol/L. Values are mean ± 1 SEM. Firm feces were scored 0 and watery feces 3.

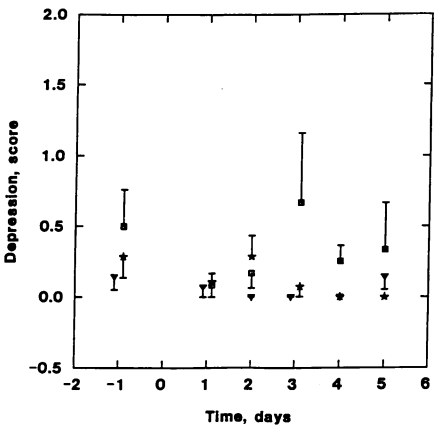

Figure 2. Severity of depression in calves receiving different oral electrolyte solutions, ▽ glycine 40 mmol/L, ★ glutamine 40 mmol/L, □ glutamine 400 mmol/L. An alert calf was scored 0, a maximally depressed calf 8. Values are mean ± 1 SEM.

Where:

Length = Length of small intestine, cm. The jejunum was assumed to account for 90% of the small intestine length, duodenum and ileum for 5% each.

Width = Width of small intestine, cm. Separate measurements were made for the duodenum, jejunum and ileum

Villi/mm^2 were calculated by counting the number of villi per high power field in a flat segment of the mucosa, dividing by the diameter of the field of view in mm and squaring the result.

Villus area, mm^2 was calculated from villus height and diameter assuming the villi to be cylinders.

The surface areas for duodenal, jejunal and ileal segments were calculated separately and then summed to give total small intestinal area. Final values are reported in m^2.

STATISTICS

An initial analysis was performed to compare the values for noninfected, control calves with those of infected, diarrheic calves. For this analysis all infected calves were treated as 1 group. Clinical and biochemical data were compared for the 1st 5 d of the control and diarrheic periods using repeated measures ANOVA.

If significant multivariate effects existed *t*-tests were performed for the individual time periods. Necropsy and xylose absorption data were compared using one way ANOVA.

For diarrheic calves, initial ANOVAs of pre-infection clinical and laboratory data were performed to determine if there were any pre-treatment differences between groups. For packed cell volume and weight data, the changes from pretreatment values were calculated for each calf. Repeated measures ANOVAs were used to look for treatment differences in the diarrheic period. If significant treatment effects existed, planned contrasts were performed to determine if differences existed between: glycine and low dose glutamine electrolyte treatment groups, and low and high dose glutamine groups. Area under the xylose absorption curve was calculated by the trapezoid method. Absorption and pathological data were analyzed using ANOVA and tukey tests. All calculations were performed using a computerized statistical package (26). P values > 0.1 are described as nonsignificant. Significant ($P < 0.05$) and intermediate ($P = 0.05$ to 0.1) P values are reported.

RESULTS

During the course of the experiment 2 diarrheic calves developed severe clinical signs and were euthanized on humane grounds. Both calves were receiving the high glutamine oral electrolyte solution. One calf died within 36 h of starting on treatment and this calf was replaced by another. The 2nd calf became progressively more dehydrated and weak on day 4 of treatment and was euthanized.

Compared to noninfected calves, infected calves had significantly higher rectal temperatures ($P < 0.01$), more diarrheic feces ($P < 0.001$), and greater hemoconcentration ($P < 0.01$). Whereas noninfected calves grew at 0.3 kg per day, infected calves lost weight at about the same rate and the differences in weight gains were significant ($P < 0.001$). Xylose absorption was halved ($P = 0.001$) and jejunal surface area reduced to 63% in infected calves ($P < 0.001$)(Table II).

Within the infected calves, there was a marginal difference in pretreatment blood pH between different treatment groups ($P = 0.09$). There were no significant differences between pre-infection values in any of the other parameters. On the 1st d of diarrhea, prior to the administration of electrolyte therapy, calves assigned to

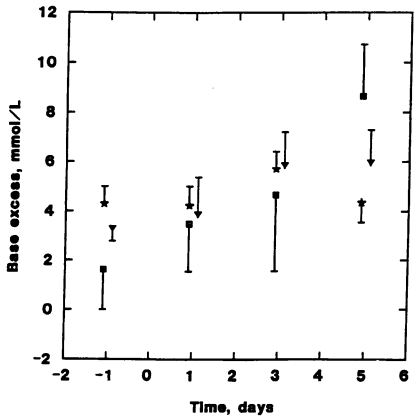

Figure 3. Severity of metabolic acidosis in calves receiving different oral electrolyte solutions, ▽ glycine 40 mmol/L, ★ glutamine 40 mmol/L, □ glutamine 400 mmol/L. Values are mean ± 1 SEM.

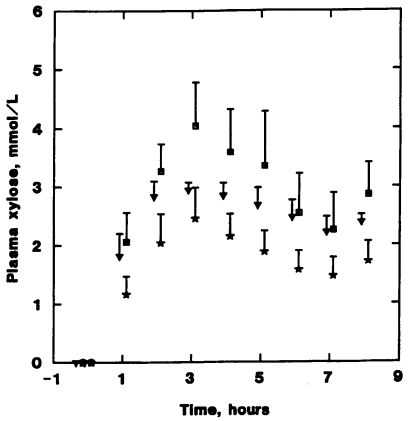

Figure 4. Plasma xylose following the oral administration of 2L of 1% xylose via nipple bottle. Electrolyte solutions contained, ▽ glycine 40 mmol/L, ★ glutamine 40 mmol/L, □ glutamine 400 mmol/L. Values are mean ± 1 SEM.

the 3 different treatment groups had similar scores for depression and severity of diarrhea which were not statistically different. Pretreatment rectal temperature on the 1st d of diarrhea did not differ between the groups ($P > 0.9$).

During the treatment phase there was a significant effect of type of oral electrolyte solution on severity of diarrhea as gauged by fecal scores ($P = 0.03$). Diarrhea was least severe in calves receiving glycine based oral electrolyte solution. After 5 d of electrolyte treatment calves receiving either low or high dose glutamine had feces that were mostly watery diarrhea. In contrast, glycine treated calves had a mean fecal score corresponding to between loose and pasty (Fig. 1). Most calves suffered either no, or only mild, depression during the trial. There was some tendency for the type of electrolyte solution administered to affect depression score (Time-electrolyte interaction $P = 0.07$). Calves receiving the high glutamine oral electrolyte were most depressed and glycine treated calves the least depressed (Fig 2). There was no significant effect (all P values > 0.20) of oral electrolyte on either clinical estimation of hydration status or change in packed cell volume. The type of electrolyte fed did not significantly affect rectal temperature (all P values > 0.2). Blood base excess was marginally different between different electrolyte treatments (time-electrolyte $P = 0.1$). At the end of the trial, calves receiving the high glutamine solution were most alkalotic (Fig 3). There was no significant effect of electrolyte treatment on blood pH (all P values > 0.2). At the beginning of the trial body weight was 42.2 ± 6.0 (mean ± SD). There was significant weight loss in all diarrheic calves (time trend $P < 0.001$) but there was no difference between treatment groups (all P values > 0.2). The area under the xylose absorption curve was not significantly different between calves on different electrolyte solutions. Although calves fed the high glutamine oral electrolyte solutions had the greatest absolute values for xylose absorption, calves receiving glycine had better absorption than those receiving equimolar amounts of glutamine (Fig 4).

At necropsy the only significant effects of different oral electrolyte treatments on gross findings were on small intestinal length ($P < 0.05$) and duodenal circumference (block-electrolyte $P < 0.05$). In both cases absolute values were greatest in calves receiving the largest amounts of glutamine (Table III). Histological measurements of duodenal villus height were significantly affected by electrolyte treatment ($P = 0.05$). Although high glutamine treated calves had the tallest villi, the more marked difference was that between the moderately long villi on the glycine treatment and the short villi of the low glutamine treated calves. There was no significant effect on any other measurement (all P values > 0.1) (Table IV). Duodenal, jejunal, ileal and total small intestinal mucosal areas were not significantly affected by electrolyte treatment. Calculated small intestinal mucosal surface area was 6.7 ± 0.7, 6.4 ± 1.0, and 5.6 ± 0.3 m^2 for glycine, high and low glutamine treatment groups respectively.

DISCUSSION

The experimental model produced a clinically relevant challenge as evidenced by the watery nature of the diarrhea in many calves and the overall 60% reduction in small intestinal mucosal surface area in diarrheic calves. Previous studies have shown that oral electrolyte solutions containing dextrose, glycine, electrolytes and alkalizing agents reduce mortality in calf diarrhea (22,24). The objective of our experiment was to determine whether replacing glycine with glutamine would further improve therapy. Although some experiments in other species show a unique role for glutamine as an energy source for intestinal metabolism (9,10,11,12) and as a beneficial nutrient in healing, our study failed to show a significant benefit to glutamine supplementation. The critical comparison when looking for a unique role for glutamine is that between the glycine and low glutamine groups. These 2 groups received equimolar amounts of amino acid. The glycine containing solution has been previously shown to be highly efficacious in preventing the development of acidosis and death in diarrheic calves (24). The experiment was designed to evaluate if replacing glycine with glutamine would further improve therapy and specifically if glutamine would improve intestinal morphology and absorption. In almost all cases where statistically significant affects existed, the data favored glycine over glutamine. The feces were more solid, the calves less depressed, the duodenal villi taller and xylose absorption was greater in the glycine rather than the low glutamine group. The glycine treated group also had the largest absolute small intestinal mucosal surface area although differences between groups

were not significant. The high glutamine treatment resembles conventional high glucose oral electrolyte solutions in energy content. Calves receiving the high glutamine solution were most depressed, had the most diarrheic feces, and had the greatest treatment failure rate. On the positive side, these calves had the longest small intestines, the highest duodenal villi and the greatest xylose absorption, although in the latter case the difference between treatments was not significant. Since the high glutamine electrolyte solution is approximately 3 times more energy dense than either the low glycine or the low glutamine solution it is possible that the greater osmotic pressure exacerbated the diarrhea and the greater energy intake helped preserve gut function rather than there being a unique effect of glutamine supplementation. Larger scale studies would be needed to confirm that the higher mortality rate on the high glutamine solution is significant.

One possible reason for the lack of a treatment effect is potential degradation of glutamine in the acid environment of the abomasum. The rise in base excess in calves receiving the highest concentration of glutamine suggests that some glutamine was absorbed, degraded to ammonia and excreted by the kidneys as ammonium ions. Urinary excretion of ammonium ions removes protons from the body, adds bicarbonate to the blood (27), and produces alkalinization and a rise in blood base excess. However, the change in base excess was relatively small and this study did not determine the fraction of administered glutamine absorbed.

Our results are consistent with those in other models of small intestinal damage. While many experiments have shown that glutamine supplementation benefits intestinal mucosal regeneration (12–17), others have not (28,29). There are various possible reasons for these differences. One is the choice of base solution. It is apparent that, although glutamine may be a preferred substrate for the mucosa, other nutrients can also be metabolized. For example, glucose and ketone bodies can be utilized by small intestinal mucosal cells (10,11). As these are metabolized via acetyl CoA it is possible that the acetate and glucose present in the base solution used in this experiment were sufficient to support mucosal energy demands. Alternatively, mucosal energy needs may have been met by the systemic circulation. Many nutrients, including glutamine, are mobilized during fasting (30) and the blood may have provided adequate glutamine for mucosal regeneration. Differences in the model studied could also account for the lack of a benefit from glutamine supplementation. Ongoing viral mediated destruction of enterocytes in our infection model may have masked mucosal regeneration. Species differences may also affect mucosal energy requirements, the substrate requirements for calf enterocytes are unknown.

A number of studies have documented glutamine facilitation of sodium and chloride absorption by enterocytes in vitro (19,21). In the present study there were no differences in clinical measures of hydration status between calves receiving glutamine or glycine containing electrolyte solutions. It is possible that glutamine could have stimulated more rapid sodium absorption than glycine based solutions. However, as the glycine containing electrolyte solution is known to be adequate to maintain hydration in the majority of diarrheic calves (24) any further increment in absorption would only result in greater urine production.

In summary, incorporation of either high or low concentrations of glutamine was of no greater benefit than the use of glycine in maintaining hydration status or supporting mucosal regeneration in diarrheic calves.

TABLE III. Gross morphological measurements (mean ± 1 SEM) in diarrheic calves treated with oral electrolyte formulations containing either glycine (40 mmol/L), high concentrations of glutamine (400 mmol/L), or low glutamine concentrations (40 mmol/L) as the amino acid source

Item	Glycine	High glutamine	Low glutamine
Crown rump length, m	94 ± 0.01	0.92 ± 0.02	0.92 ± 0.03
Small intestine length, m	15.8 ± 0.52	18.6 ± 0.92	17.4 ± 0.50
Small intestine weight (empty), g	873.4 ± 67.2	908.9 ± 89.4	821.7 ± 31.8
Duodenal circumference, cm	3.4 ± 0.18	3.4 ± 0.15	3.3 ± 0.06
Jejunal circumference, cm	3.4 ± 0.26	3.2 ± 0.17	3.3 ± 0.12
Large intestine length, m	2.11 ± 0.11	2.15 ± 0.14	2.25 ± 0.09
Large intestine weight (empty), g	293.8 ± 19.2	279.0 ± 31.2	286.6 ± 9.0

TABLE IV. Histological measurements (mean ± 1 SEM) in diarrheic calves treated with oral electrolyte formulations containing either glycine (40 mmol/L), high concentrations of glutamine (400 mmol/L), or low glutamine concentrations (40 mmol/L) as the amino acid source

Item	Glycine	High glutamine	Low glutamine
Duodenal villus height, mm	0.588 ± 0.066	0.613 ± 0.088	0.461 ± 0.049
Duodenal crypt depth, mm	0.278 ± 0.019	0.295 ± 0.034	0.270 ± 0.010
Jejunal villus height, mm	0.542 ± 0.070	0.443 ± 0.059	0.459 ± 0.062
Jejunal crypt depth, mm	0.309 ± 0.023	0.299 ± 0.038	0.287 ± 0.020
Ileal villus height ,mm	0.417 ± 0.029	0.410 ± 0.012	0.370 ± 0.015
Ileal crypt depth,mm	0.266 ± 0.021	0.260 ± 0.010	0.253 ± 0.012
Proximal colon ridge height, mm	0.541 ± 0.028	0.548 ± 0.014	0.536 ± 0.034
Distal colon ridge height, mm	0.573 ± 0.024	0.592 ± 0.022	0.528 ± 0.020

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