2018, Scienceline Publication
World's Veterinary Journal
World Vet J, 8(2): 34-47, June 25, 2018
ISSN 2322-4568

Management of Short Bowel Syndrome in Nigerian Dogs

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ABSTRACT

The effects of glutamine, honey, ascorbic acid and glutamine, honey, ascorbic acid combination on small bowel adaptation following 70% small intestinal resection in local Nigerian dogs were investigated. Thirty adult dogs with a median weight of 12.4kg (range 7-18 kg) were used in this study. The dogs were randomized into five groups of six dogs each following resection. Group 1 is the control group. The dogs here were not placed on any treatment. Group 2 dogs were placed on glutamine. Group 3 dogs were placed on honey. Group 4 dogs were placed on ascorbic acid and group 5 dogs were placed on glutamine, honey and ascorbic acid combination. Intestinal biopsy samples were collected at day 0, 4 weeks, 6 weeks and 8 weeks for histomorphometric study. Intestinal morphology was evaluated using light microscopy. The body weights of the dogs were monitored weekly for 4, 6 and 8 weeks in the five groups. Small intestinal adaptive response was evaluated at 4, 6 and 8 weeks post surgery. The animals treated with glutamine, honey, ascorbic acid and glutamine/ honey/ ascorbic acid combination experienced increase in intestinal villus height, villus width, crypt depth and wall thickness. The control group experienced a fall in intestinal villus height, villus width, crypt depth and wall thickness. The animals treated with glutamine, honey, ascorbic acid combination showed better therapeutic response followed by glutamine, honey and ascorbic acid in that order. There was a gradual increase in the body weight of animals in these groups. The control group did not show any appreciable adaptive response and the animals in this group progressively lost weight. It was concluded that dogs presented with short bowel syndrome could benefit from the supplementation of glutamine, honey and ascorbic acid in food.

Key words: Short bowel syndrome, Total parenteral nutrition, Intestinal resection, Small intestinal adaptive response

ORGINAL ARTICLE
pii: S2322456818000004-8
Received: 04 Apr 2018
Accepted: 08 May 2018

INTRODUCTION

Extensive resection of the small intestine can result in a condition known as Short Bowel Syndrome (SBS) (Gorman et al., 2006; Hasosah et al., 2008; Seetharam and Rodrigues, 2011; Mayeur et al., 2016; Vagholkar et al., 2016) and it occurs in both humans and animals. It is a disorder characterized by an intestinal absorptive surface area that is insufficient to support the host. This intestinal loss results in the malabsorption of fluid, electrolytes and other essential nutrients, severe diarrhea, dehydration and progressive malnutrition. Intestinal failure results when the residual bowel length cannot meet the patient's nutritional requirement thus necessitating dependence on parenteral nutrition (PN) (Donohoe and Reynolds, 2010; Norholk et al., 2012; Winkler and Smith, 2014; Mayer and Kerner, 2017). It is this dependence on parenteral nutrition that is responsible for the majority of morbidity and mortality associated with short bowel syndrome (Wales and Christison-Lagay, 2010; Vipperla and O'keefe, 2014). In dogs, intestinal resection is done because of linear foreign bodies, mesenteric volvulus, direct traumatic damage to the small bowel wall, solitary foreign body with perforation of the intestine, intussusceptions, dehiscence of a previous gastrointestinal tract surgery site and gastrointestinal tract tumor (Gorman et al., 2006). Long-term survival of patients with SBS is dependent on the adaptation of the remaining small intestine and response to pharmacological and nutritional management (Wall, 2013, Cunha-Melo and Costa, 2014; Rodriguez-Montes et al., 2016).

Managing short bowel syndrome is challenging as the medications are expensive and patients have to use them for a very long time and there is no guarantee that the response will be favourable (Hrbaty et al., 2004).

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MATERIALS AND METHODS

Experimental animals

Thirty dogs under 2 years of age with a mean weight of 12.4 kg (range 7-18 kg) were used for this study. They were stabilized for 4 weeks by being boarded in kennels within the teaching hospital and were dewormed and treated against ectoparasites and hemoparasites. They were fed daily and water was provided ad libitum. Each animal was fasted for 12 hours prior to surgery. They were premedicated with Atropine sulphate (Jiangsu Huayang pharmaceutical, China) at a dose rate of 0.04 mg/kg and xylazine hydrochloride (XYL-M2®, VMD, Belgium) at a dose rate of 1 mg/kg body weight intramuscularly. Induction was done with thiopentone sodium (Rotexmedica, Germany) at a dose rate of 10 mg/kg body weight intravenously. The total small intestinal length in each dog was calculated and recorded as described by Kisani et al. (2017).

Surgical procedure

Each animal was aseptically prepared and a ventral midline abdominal incision was made. The intestinal tract was exteriorized. Seventy per cent of the small intestinal tract (from the result of the preliminary study) was resected beginning from a point 7cm from the duoduno-jejunal flexure (treitz ligament). The residual intestinal tract was sutured using end to end anastomosis with polyglactin 910 (vicryl® Ethicon, USA) size "0" as described earlier. A full thickness biopsy sample of the small intestinal tract (Jejunum and ileum) were collected and fixed in 10% formalin (pretreatment sample). The anastomostic site was covered with omentum and returned to the abdominal cavity. The abdominal incision was closed using standard surgical technique (Fossum, 2014). Procain penicillin (Shuazhuang co ltd, China) 20,000 iu/kg and Streptomycin (North China pharmaceutical co ltd, China)(10 mg/kg) was administered intramuscularly for 5 days post operative. Pentazocin (Bharat Parenterals ltd, India) was administered intramuscularly at the dose rate of 5mg/kg for 7 days to relieve pain.

The dogs were given 5% dextrose infusion intravenously at 10 mls/kg/hr on the second and third day post operative. They were fed bland diet gruel on the fourth post operative day and returned to normal solid diet on day five post surgery. After surgery, the animals were randomized into five groups that each group contained six animals. Group 1 was the control group. The animals in this group were not treated. They were left to attend to their normal daily ration throughout the period of experiment. Group 2: Animals in this group were placed on orally administered glutamine. The dogs were given 9.0 mg/kg/day for 4 weeks (two dogs), 12.0 mg/kg/day for 6 weeks (Two dogs) and two15.0 mg/kg/day for 8 weeks (two dogs).

Group 3: The animals in this group were placed on orally administered honey. Two dogs were placed on honey at 7.5 mls/day for 4 weeks, two dogs consumed10mls/day for 6 weeks and two dogs were administered 12.5 mls/day for 8 weeks. Group 4: The animals in this group were placed on orally administered Ascorbic acid (vitamin C). Two dogs were administered ascorbic acid 150 mg/day for 4weeks, two dogs administered on ascorbic acid 250mg/day for 6 weeks and two dogs administered 350 mg/day for 8 weeks. Group 5: The animals in this group were placed on combine dose of glutamine (9 mg/kg/day), honey (7.5 mls/day) and vitamin C (150 mg/day). Two animals were placed on this combination for 4 weeks, two animals administered for 6weeks and two animals administered for 8 weeks.

Histomorphometric analysis

Small bowel biopsy specimens were obtained from the jejunum and ileum at day 0 (pre-surgical) and at 4, 6 and 8 weeks post resection-anastomosis. A total of four biopsy specimens were obtained from each patient (at the end of first and second laparotomy) and placed in 10% formalin. After routine formalin fixation, biopsy specimens were embedded in paraffin. Four-micrometer-thick (4 µm) sections were cut perpendicular to the mucosa and stained routinely with H&E. Then using light microscopy and an eye piece micrometer at a magnification of ×100, the villus height, villus width, crypt depth and cross sectional area of the bowel mucosa were measured as explained by Joaquim et al. (2005). Five readings were taken for each parameter and the average value determined. Photomicrograph and interpretation of the slides were done using trinocular microscope and Amscope Toup View 3.7.

Statistical analysis

Data were expressed as descriptive statistics. Differences among the groups were evaluated using one way analysis of variance (ANOVA) followed by a two tailed student's t-test using SPSS version 16. The level of significance was set at 5%.

Ethical approval

This study was approved by the ethical committee of the college of veterinary medicine, university of Agriculture, makurdi, Nigeria with no.001.

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RESULTS

Histomorphometry

Villus height

There was an increase in villus height for all the groups except the control group which experienced a fall in jejuna villus height in week 6 and 8 (Table 1) and a fall in ileal villus height in week 4 and 6. Group 5 also experienced a fall in jejuna villus height in week 6 (Table 5). The animals in group 4 showed a fall in ilea villus height in week 6. Groups 2, 3, and 5 showed statistically significant increase in villus height (Tables 2, 3 and 5) ($P<0.05$) while groups 1 and 4 did not cause statistical increase in villus height (Tables 1 and 4) ($P>0.05$).

Villus width

There was increase in villus width in all the groups. The control group and glutamine treated groups had greater increase in jejuna villus width at week 4 and a decrease in week 6 and 8 (Table 1). The control group also experienced a decrease in ilea villus width in week 4 and 6. Glutamine, honey and ascorbic acid treated groups had shown a statistically significant increase in villus width (Tables 2, 3 and 4) ($P<0.05$). Ascorbic acid treated group had also shown significant increase in ilea villus height.

Crypt Depth

There was an increase in crypt depth in all the groups except the control group (Tables 1-5). Glutamine, honey and glutamine honey and ascorbic acid treated groups caused a significant increase in crypt depth ($P<0.05$). Ascorbic acid treated animals significantly enhanced ilea crypt depth while the control group did not show any significant changes.

Wall thickness

All the groups experienced increase in wall thickness except the control group which experience increase only in the ilea wall thickness in week 8 of experiment (Tables 1-5). Group 2 (glutamine treated), group 3 (honey treated) and group 5 (glutamine/ honey/ ascorbic acid treated) animals had shown a significant increase in wall thickness ($P< 0.05$) Tables 2, 3 and 5). Ascorbic acids treated group had shown a significant increase in jejunal wall thickness ($P<0.05$) (Table 4).

Table 1. Jejunal histomorphometric analysis for dogs (Control group) post 70% small intestinal resection

Jejunum	WKs	Number of dogs (n = 6)						Mean ±SEM	P value
		1	2	3	4	5	6		
Villus Height (VH)	0	1.104	0.937	0.696	0.787	0.814	0.925	0.877±0.058	0.131
	4	1.024	0.861	-	-	-	-	0.943±0.082	
	6	-	-	0.638	0.725	-	-	0.682±0.044	
	8	-	-	-	-	0.772	0.567	0.670±0.103	
Villus Width (VW)	0	0.979	0.876	0.166	0.192	0.177	0.140	0.422±0.161	0.138
	4	0.908	0.796	-	-	-	-	0.852±0.056	
	6	-	-	0.110	0.132	-	-	0.121±0.011	
	8	-	-	-	-	0.145	0.102	0.124±0.022	
Crypt Depth (CD)	0	0.426	0.322	0.680	0.799	0.565	0.322	0.519±0.080	0.216
	4	0.357	0.248	-	-	-	-	0.303±0.055	
	6	-	-	0.622	0.744	-	-	0.683±0.061	
	8	-	-	-	-	0.528	0.282	0.405±0.123	
Wall Thickness (WT)	0	1.250	0.840	1.021	0.765	0.918	0.826	0.937±0.072	0.812
	4	1.038	0.597	-	-	-		0.818±0.221	
	6	-	-	0.961	0.699	-	-	0.830±0.131	
	8	-	-	-	-	0.887	0.795	0.841±0.046	

WKs= Weeks; n= Number of dogs

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Table 2. Jejunal histomorphometric analysis for dogs post 70% small intestinal resection

Jejunum	WKs	Number of dogs (n = 6)						Mean ±SEM	P value
		1	2	3	4	5	6		
Villus Height (VH)	0	0.753	0.876	0.922	0.730	0.684	0.779	0.791±0.037	0.0002
	4	1.026	1.162	-	-	-	-	1.094±0.068	
	6	-	-	1.317	1.117	-	-	1.217±0.100	
	8	-	-			1.341	1.431	1.386±0.045	
Villus Width (VW)	0	0.141	0.156	0.133	0.129	0.143	0.138	0.422±0.161	0.138
	4	0.182	0.602	-	-	-	-	0.852±0.056	
	6	-	-	0.204	0.197	-	-	0.121±0.011	
	8	-	-	-	-	1.068	1.029	0.124±0.022	
Crypt Depth (CD)	0	0.555	0.676	0.492	0.472\	0.394	0.478	0.511±0.039	0.0037
	4	0.606	0.731	-	-	-	-	0.669±0.063	
	6	-	-	0.794	0.768	-	-	0.781±0.013	
	8	-	-	-	-	0.805	0.896	0.851±0.046	
Wall Thickness (WT)	0	0.580	0.767	0.804	0.814	0.961	0.607	0.756±0.058	0.0003
	4	0.815	0.999	-	-	-	-	0.907±0.092	
	6	-	-	1.152	1.168	-	-	1.160±0.008	
	8	-	-	-	-	1.922	1.559	1.741±0.182	

WKs= Weeks; n= Number of dogs

Table 3. Jejunal histomorphometric analysis for dogs pre and post 70% small intestinal resection and anastomosis

Jejunum	WKs	Number of dogs (n = 6)						Mean ±SEM	P value
		1	2	3	4	5	6		
Villus Height (VH)	0	0.866	0.940	0.879	0.761	1.230	0.720	0.899±0.074	0.042
	4	1.047	1.102					1.075±0.028	
	6			1.173	1.073			1.123±0.050	
	8					1.631	1.124	1.378±0.254	
Villus Width (VW)	0	0.152	0.126	0.163	0.134	0.192	0.141	0.151±0.010	0.030
	4	0.190	0.158					0.174±0.016	
	6			0.214	0.183			0.199±0.016	
	8					0.249	0.201	0.225±0.024	
Crypt Depth (CD)	0	0.558	0.708	0.658	0.620	0.774	0.490	0.635±0.042	0.027
	4	0.589	0.742					0.666±0.077	
	6			0.870	0.920			0.895±0.025	
	8					1.084	0.802	0.943±0.141	
Wall Thickness (WT)	0	0.636	0.800	0.798	0.669	0.724	0.972	0.767±0.049	0.0001
	4	0.787	0.984					0.886±0.099	
	6			1.047	0.881			0.964±0.083	
	8					1.636	1.834	1.735±0.099	

WKs= Weeks, n= Number of dog

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Table 4. Jejunal histomorphometric analysis for dogs pre and post 70% small intestinal resection and anastomosis

Jejunum	WKs	Number of dogs (n = 6)						Mean ±SEM	P value
		1	2	3	4	5	6		
Villus Height (VH)	0	0.873	0.796	0.992	0.693	0.694	0.772	0.803±0.047	0.093
	4	0.994	0.972					0.983±0.011	
	6			1.131	0.872			1.014±0.138	
	8					1.039	1.113	1.076±0.037	
Villus Width (VW)	0	0.114	0.163	0.134	0.174	0.153	0.137	0.146±0.009	0.132
	4	0.132	0.178					0.155±0.023	
	6			0.166	0.210			0.188±0.022	
	8					0.197	0.178	0.188±0.010	
Crypt Depth (CD)	0	0.790	0.438	0.702	0.511	0.545	0.603	0.598±0.053	0.356
	4	0.81	0.456					0.633±0.177	
	6			0.842	0.691			0.767±0.076	
	8					0.747	0.807	0.777±0.030	
Wall Thickness (WT)	0	0.700	0.840	0.629	0.608	0.588	0.494	0.643±0.048	0.005
	4	0.776	0.924					0.850±0.074	
	6			0.979	0.928			0.954±0.026	
	8					1.091	0.993	1.042±0.049	

WKs= Weeks; n= Number of dog

Table 5. Jejunal histomorphometric analysis for dogs pre and post small intestinal resection and anastomosis

Jejunum	WKs	Number of dogs (n = 6)						Mean ±SEM	P value
		1	2	3	4	5	6		
Villus Height (VH)	0	1.333	0.981	0.788	0.765	1.080	0.965	0.985±0.085	0.002
	4	1.646	1.298					1.472±0.174	
	6			1.318	1.305			1.312±0.007	
	8					1.941	1.821	1.881±0.060	
Villus Width (VW)	0	0.135	0.127	0.132	0.164	0.130	0.152	0.140±0.006	0.0001
	4	0.191	0.187					0.189±0.002	
	6			0.212	0.265			0.239±0.027	
	8					0.271	0.321	0.296±0.025	
Crypt Depth (CD)	0	0.574	0.961	0.413	0.592	0.536	0.882	0.660±0.087	0.070
	4	0.639	1.023					0.831±0.192	
	6			0.812	0.997			0.905±0.093	
	8					1.05	1.402	1.226±0.176	
Wall Thickness (WT)	0	0.716	0.786	0.890	0.597	0.882	0.943	0.802±0.053	0.0001
	4	1.100	1.175					1.138±0.038	
	6			1.355	1.053			1.204±0.151	
	8					1.912	1.994	1.953±0.041	

WKs= Weeks; n= Number of dog

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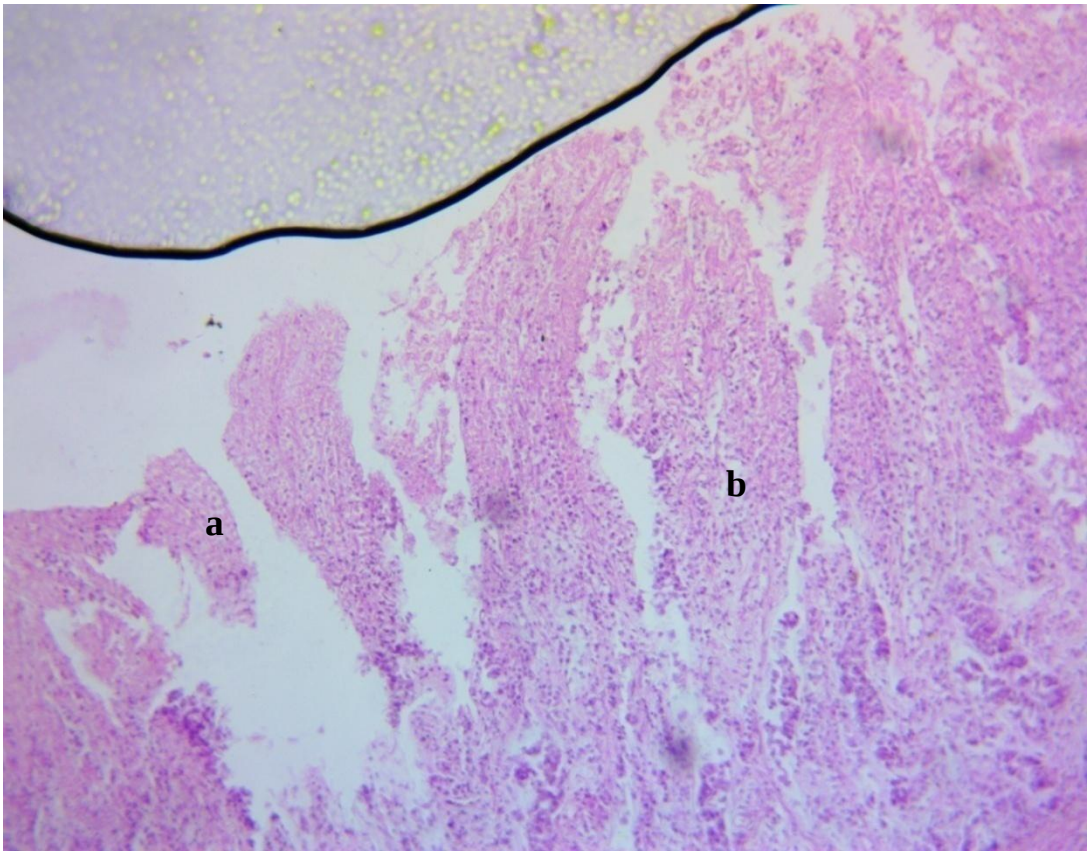

Figure 1. The jejunum of dogs at eight weeks post resection showing (a) Necrosis of villi and (b) leucocytes infiltration of laminal propria. (H&E) ×100

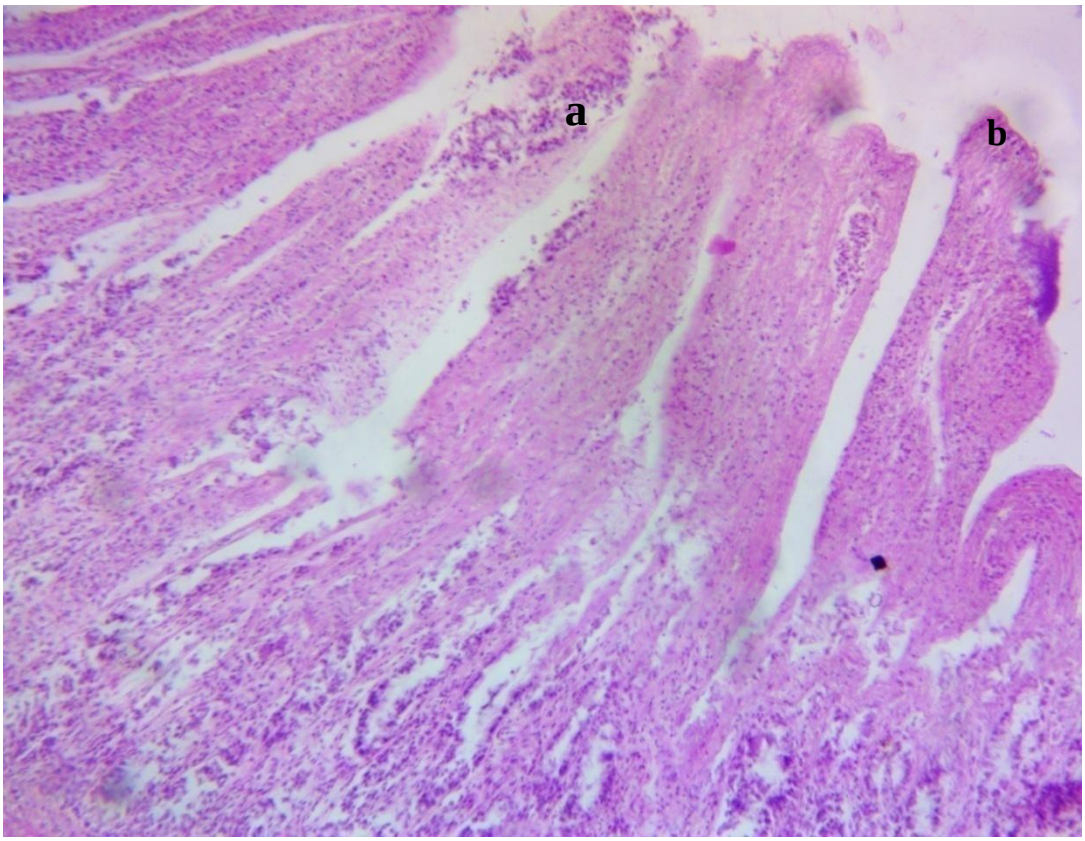

Figure 2. The Ileum of dogs at eight weeks post resection showing (a) mild necrosis and (b) moderate hyperplasia of villi (H&E) ×100

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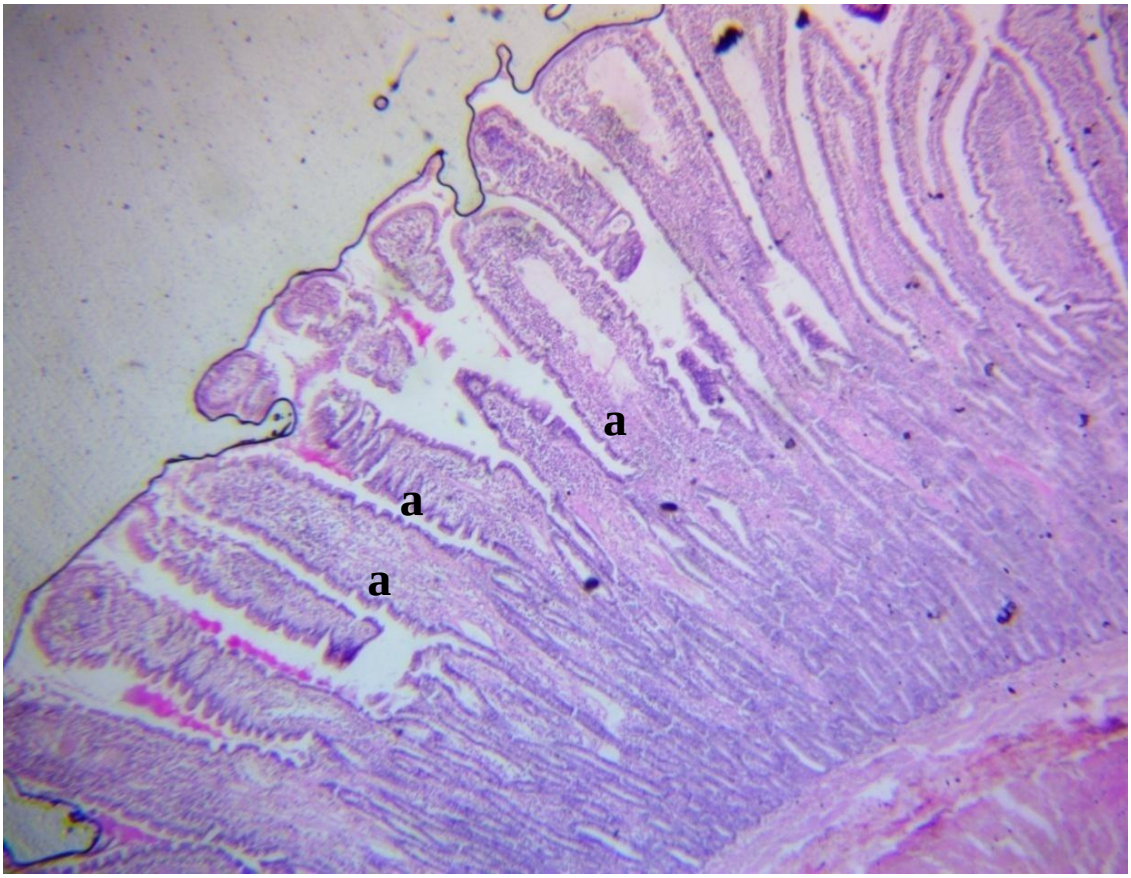

Figure 3. The Jejunum of dogs at eight weeks after treatment with glutamine showing (a) formation of surface epithelium (H&E) ×40

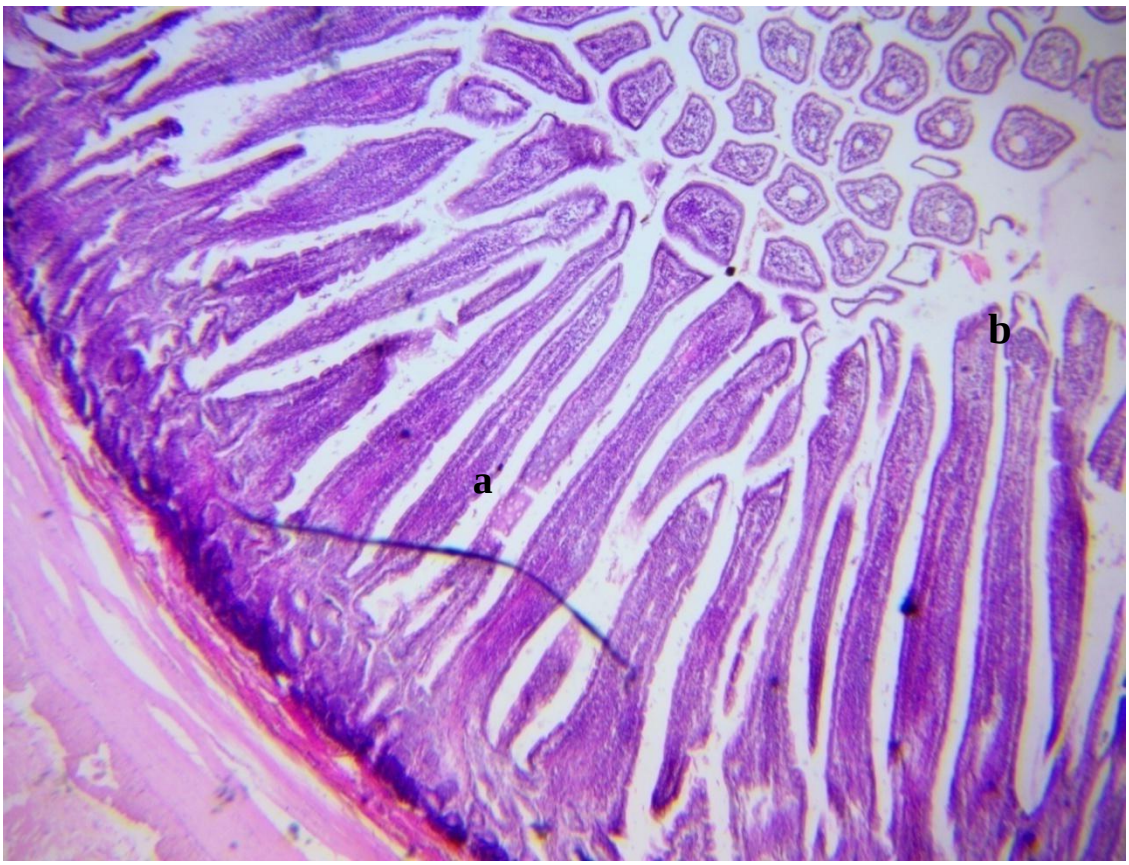

Figure 4. The ileum of dogs at eight weeks after treatment with glutamine showing (a) surface epithelium (b) Hyperplastic villi (H&E) ×100

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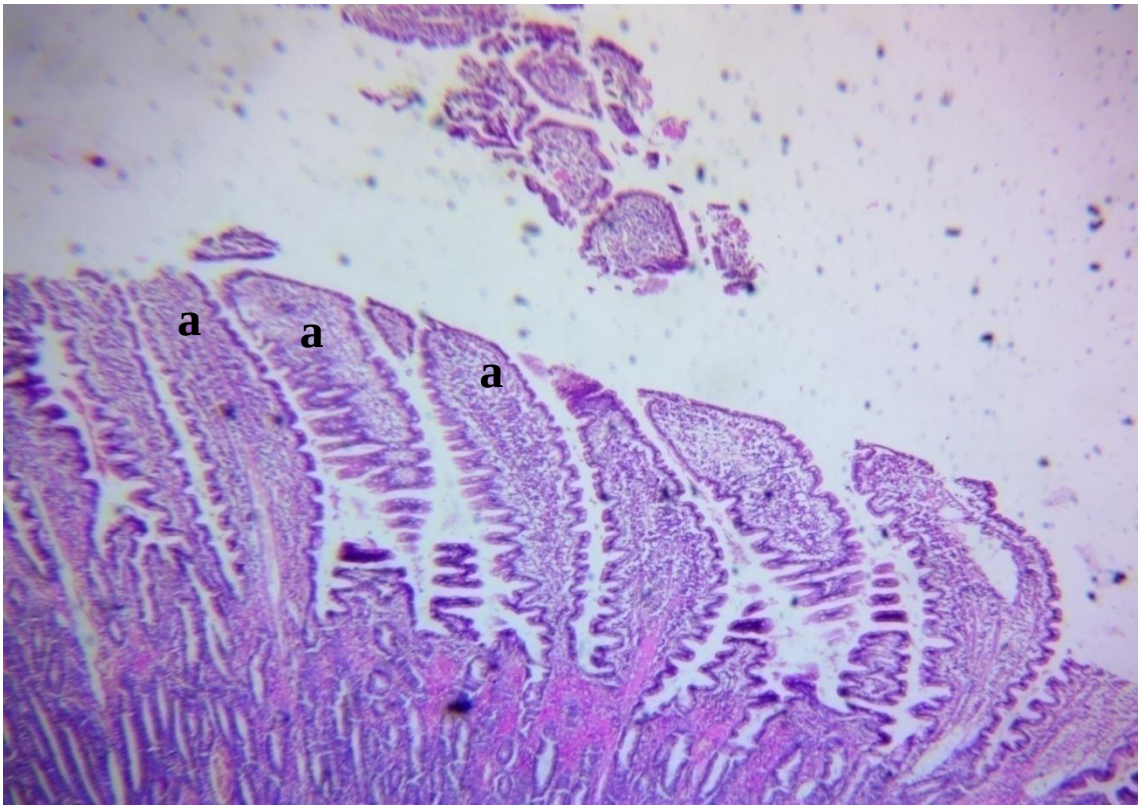

Figure 5. The Jejunum of dogs at eight weeks after treatment with honey showing normal villi – "a" (H&E) ×40

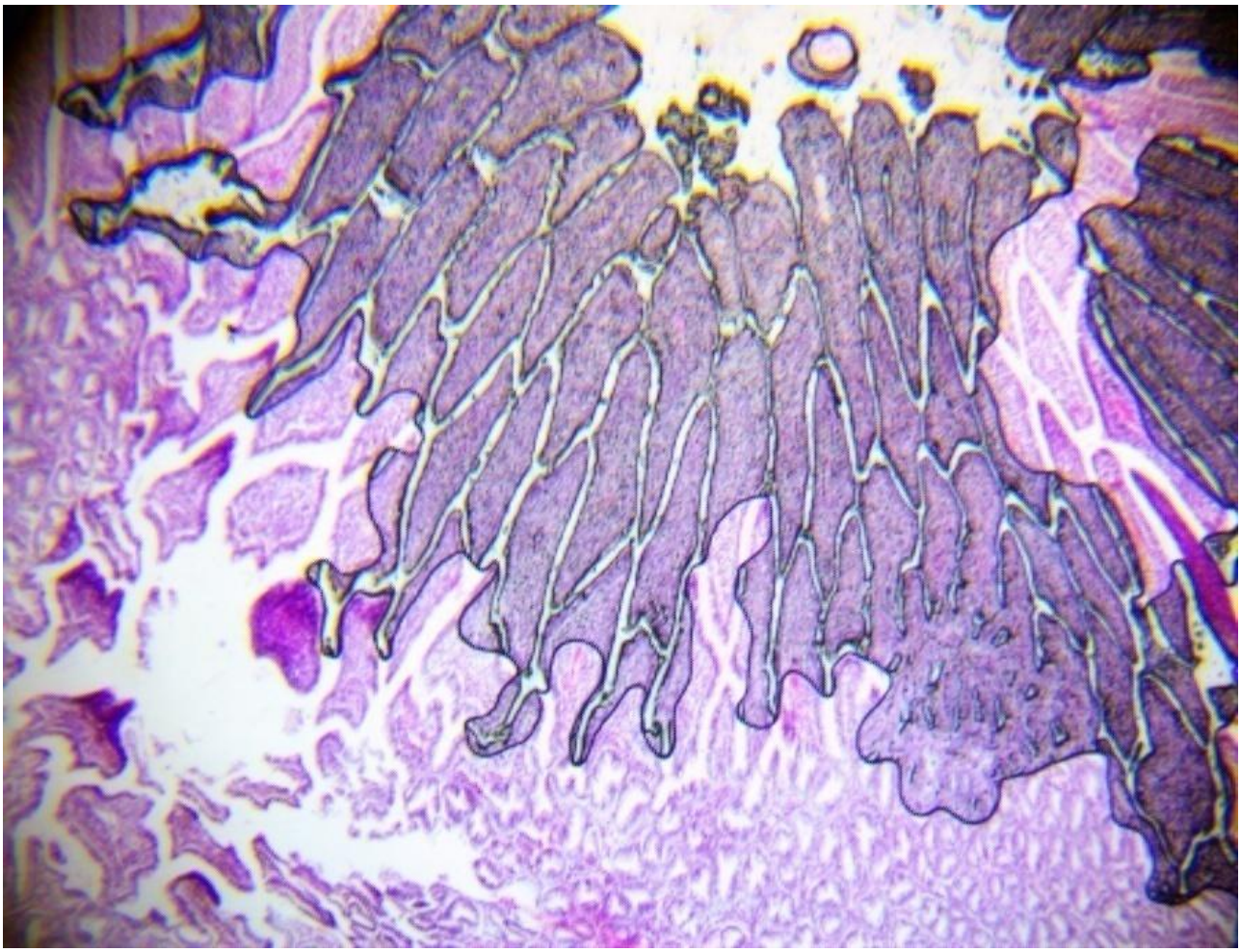

Figure 6. The ileum of dogs at eight weeks after treatment with honey showing complete recovery (H&E) ×40

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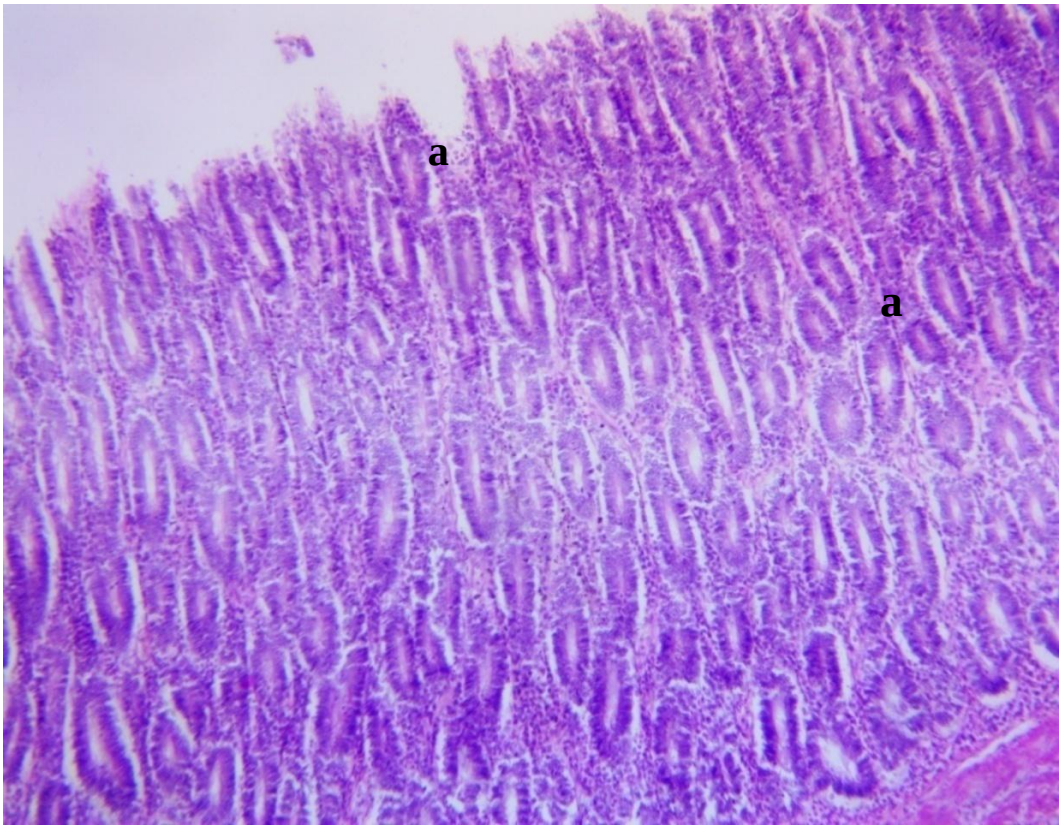

Figure 7. The Jejunum of dogs at eight weeks after treatment with ascorbic acid showing leucocytic infiltration of lamina propria of the intestinal mucosa (a) (H&E) ×100

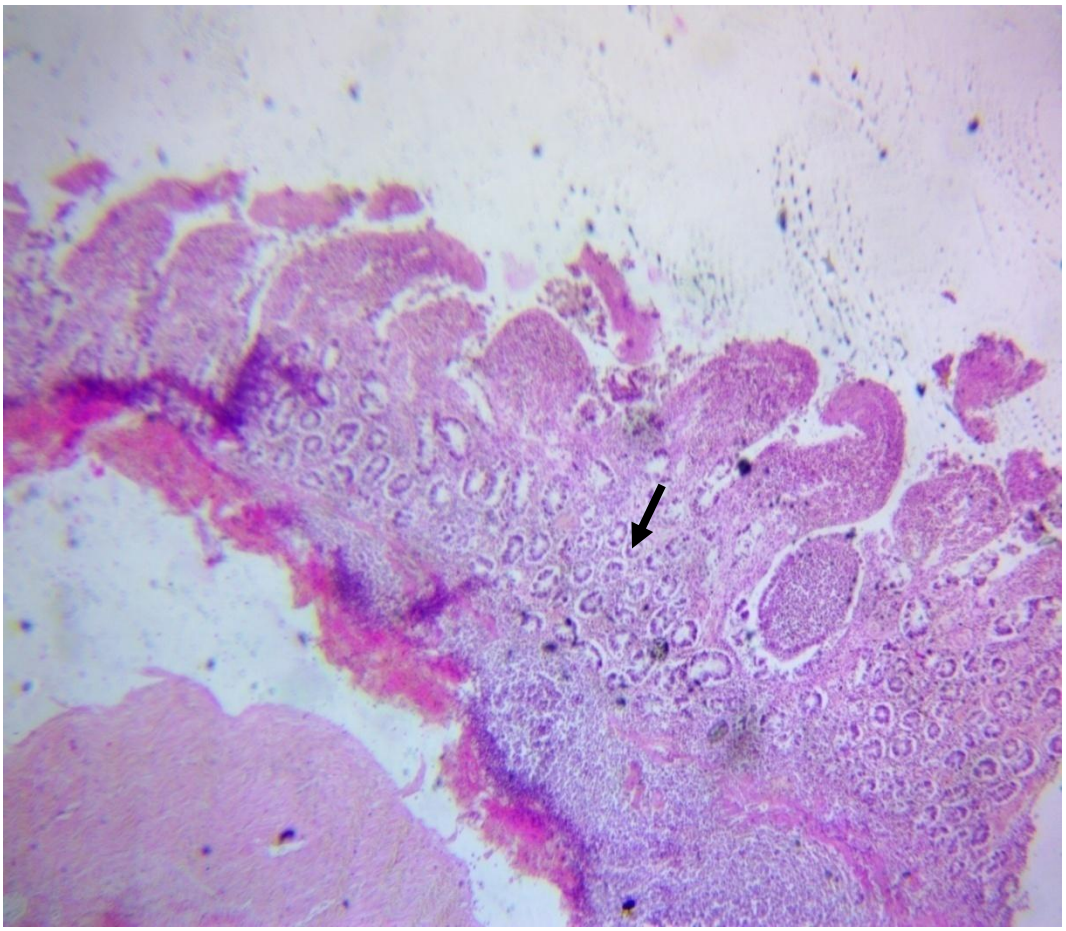

Figure 8. The ileum of dogs at eight weeks after treatment with ascorbic acid showing thickened lamina propria infiltrated by leucocytes (arrow) (H&E) ×40

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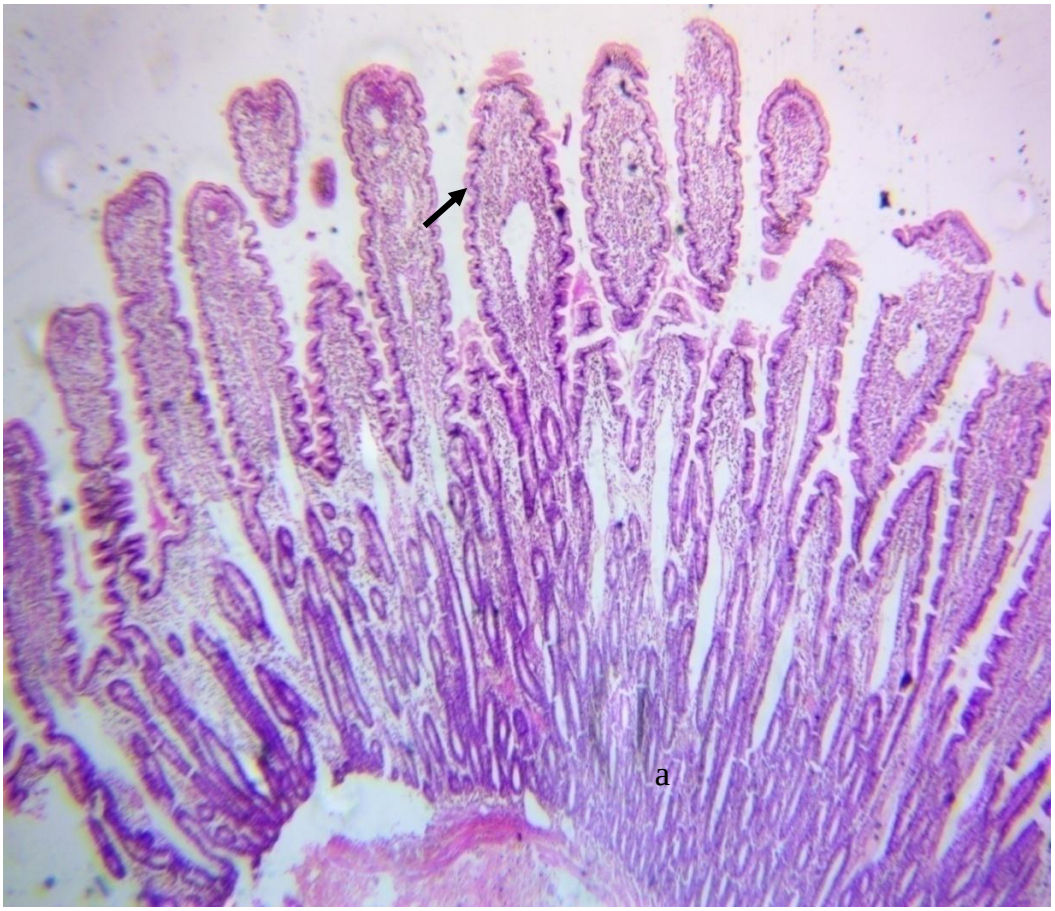

Figure 9. The Jejunum of dogs at eight weeks after treatment showing well formed villi (arrow) (H&E) ×40

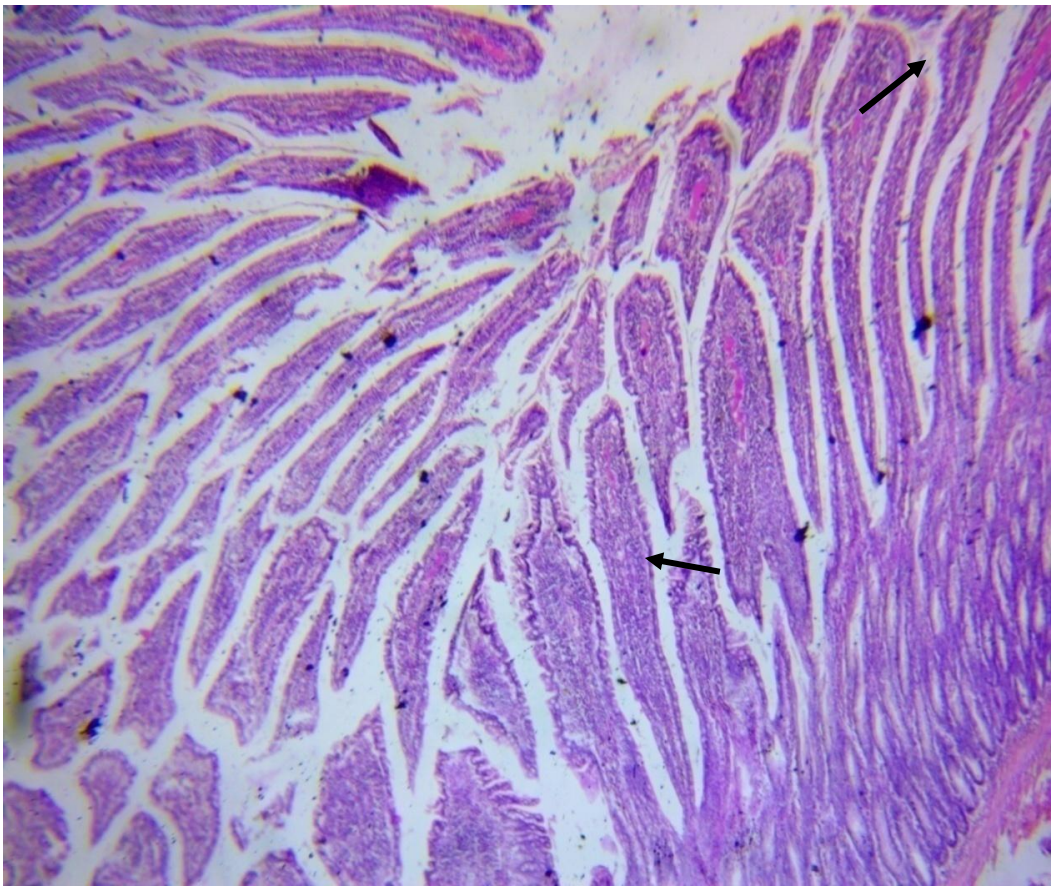

Figure 10. The Ileum of dogs at eight weeks after treatment showing well formed surface epithelium and villi (arrow) (H&E) ×40

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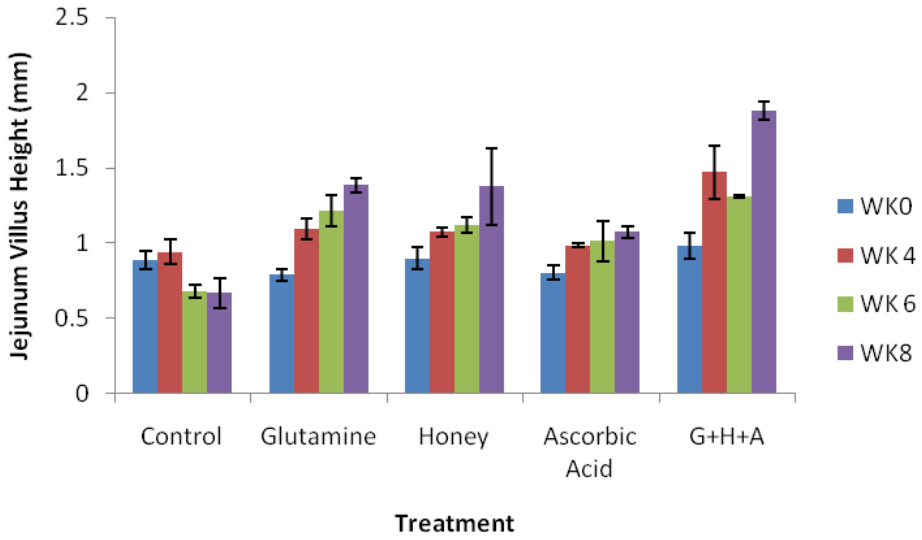

Diagram 1. Pre-resection and post-treatment jejunal villi height of dogs in control, glutamine, honey, ascorbic acid and glutamine/ honey/ ascorbic acid combination treated groups

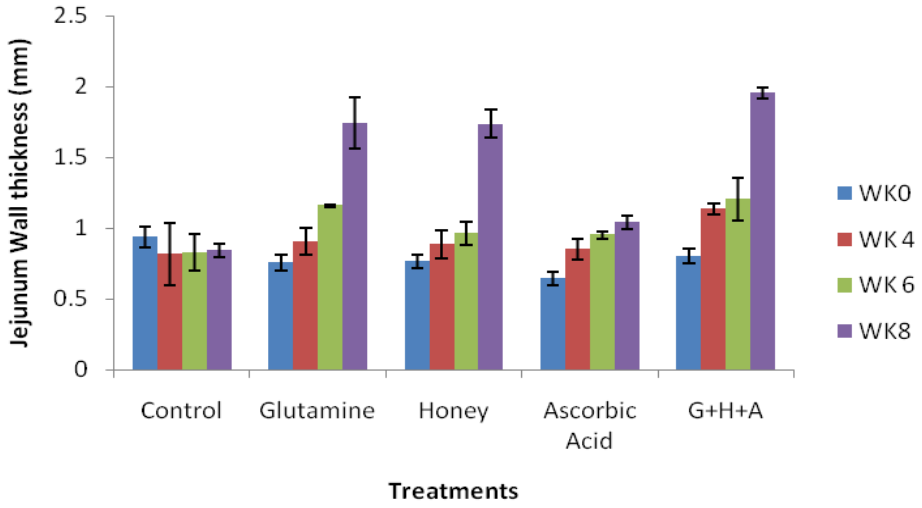

Diagram 2. Pre-resection and post-treatment jejuna wall thickness of dogs in control, glutamine, honey, ascorbic acid and glutamine/ honey / ascorbic acid combination treated groups

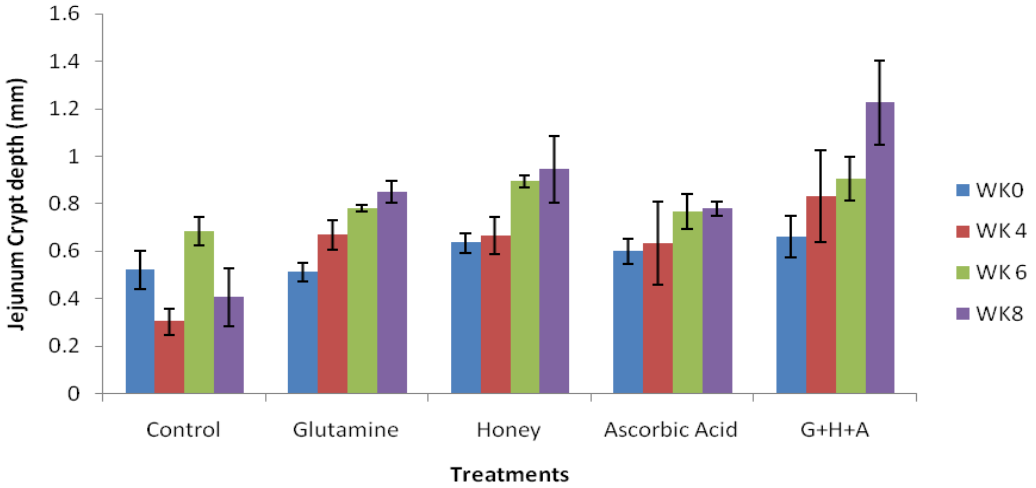

Diagram 3. Pre-resection and post-resection jejunal crypt depth of dogs in control, glutamine, honey, ascorbic acid and glutamine/ honey/ascorbic combination acid treated groups

To cite this paper: Kisani AI, Adeyanju JB, Elsa AT and Sonfada ML (2018). Management of Short Bowel Syndrome in Nigerian Dogs. *World Vet. J.* 8(2): 34-47.
Journal homepage: www.wvj.science-line.com

DISCUSSION

In present study, the effects of glutamine, honey, ascorbic acid and glutamine, honey and ascorbic acid combination on intestinal adaptation in Nigerian dogs suffering from short bowel syndrome following 70% small bowel resection was evaluated. Dogs in all the groups in this study had diarrhea and loss of weight which is in agreement with the findings of Gorman et al. (2006), Shaw et al. (2012) and Tappenden (2014). The reason was due to mal-absorption as a result of the loss of mucosal absorptive surface area associated with short bowel syndrome (Shaw et al., 2012; Cunha-Melo and Costa, 2014; Merritt et al., 2017).

However, there was a reduction in diarrhea in animals treated with glutamine, honey, ascorbic acid and their combination after one week of treatment. This is due to the adaptive changes taking place in the intestinal mucosa that improves absorption and digestion of nutrients (Shaw et al., 2012; Vagholkar et al., 2016; Gillard et al., 2017). This is the reason for the increase in weight observed in animals in these four groups.

Animals in the control group however, continue to experience diarrhea especially after feeding due to inadequate adaptive changes in the intestinal mucosal. Hence the gradual drop in body weight observed in this group. Glutamine is the major nutrient for the bowel. It is the primary substrate used by enterocytes. Supplementation during catabolic states improves structural integrity, function and repair of the intestinal mucosa, decreases bacterial translocation and epithelial apoptosis, improves nitrogen balance and influencing gut immune response (Chandler, 2002; Evans et al., 2003; Grimble, 2005; Larson et al., 2007). Dogs treated with glutamine (group 2) experienced significant increase ($P<0.05$) in Villus Height (VH), Crypt Depth (CD), Villus Width (VH) and Wall Thickness (WT). The gradual increase in body weight observed in this group may be due to enhanced absorption of nutrients from improved adaptation of the remnant small bowel. Our findings on the use of glutamine in this study, agrees with the previous work by Eyarefe et al. (2012), who used glutamine on dogs with short bowel syndrome following massive small bowel resection and observed a beneficial adaptive response by the remnant small bowel in those dogs. In this study, dogs treated with honey (group 3) showed a significant increase ($P<0.05$) in VH, CD, VW and WT. There was a gradual increase in body weight. There was also a complete regeneration of the intestinal epithelium. This result is also in agreement with the findings of Eyarefe et al. (2012) who also used honey to treat dogs suffering from short bowel syndrome and observed positive adaptive response.

Dogs treated with ascorbic acid showed marginal increase in VH, VW, CD and WT. It has been reported that malassimilation and increased fluid losses with diarrhea may deplete vitamin C in the body (Chandler, 2002). It may be possible that the dosage of vitamin C used in this study was not enough to compensate for the large losses that had occurred. Though the increase was not statistically significant, it was comparatively better than that of the control group. The action of ascorbic acid (vitamin C) was as a result of its powerful antioxidant property where it scavenges and mopes up reactive oxygen and nitrogen species (free radicals) in the body. These reactive species are generated by normal cell processes as well as environmental stressors and can cause damage to tissues (Halliwell and White man, 1997; Telang, 2013). Oxidative stress also occurred in the intestine in diseases and may cause increased inflammation and permeability (Chandler, 2002). Thus vitamin C reduced potential damage to tissues. All these positive effects may probably be undermined in this study, due to the insufficiency of vitamin C, which may be responsible for the low/gradual villi height, crypt depth, villi width and wall thickness. The evaluation of ascorbic acid in the treatment of short bowel syndrome did not report experimentally and there are not enough published data about this research topic.

Dogs on glutamine, honey and ascorbic acid combination (group 5) showed significant increase in VH, VW, CD and WT. This adaptive response was higher than that observed in glutamine (group 2), honey (group 3) or ascorbic acid (group 4) treated animals respectively. This showed that the combination of these three supplements had synergistic effect that could enhance small bowel adaptation in patients with short bowel syndrome. Similarly, the synergistic effect of these products has not been reported. Animals in the control group did not show any appreciable adaptive response as there was no significant increase in VH, VW, CD and WT. There was also drop in body weight.

The result of this study has revealed that glutamine, honey and their combination have beneficial intestinal adaptive response in patients suffering from short bowel syndrome. However, /glutamine/ honey/ ascorbic acid combination produces higher adaptive response than glutamine, honey or ascorbic acid alone. The higher doses of these supplements produced better adaptive response than the lower doses.

CONCLUSION

Glutamine, honey and ascorbic acid given individually or in combination enhanced residual bowel efficiency and improved outcome in patients suffering from short bowel syndrome. It is therefore recommended that dogs with signs attributable to short bowel syndrome should be given supplement of glutamine, honey and ascorbic acid in food.

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DECLARATIONS

Author's contribution

AIK, JBA and ATE performed the surgery. MLS prepared the histopathology slides and reviewed the manuscript.

Competing interests

The authors have declared that no competing interest exists

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