MEASUREMENT OF RABBIT'S INTESTINAL VILLUS: PRELIMINARY COMPARISON OF TWO METHODS

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ABSTRACT

This work applies the microdissection technique, described by Clarke (1977), and the conventionally prepared sections stained with haematoxilin and eosin to samples from rabbit small intestine. Twenty-four rabbits, previously brooded under identical conditions with a basal diet were slaughtered at 24 (n=8), 32 (n=8) and 42 (n=8) days of age to collect samples for laboratory analysis. Two adjacent sections of ileum were collected, one was fixed in 10% neutral formalin for paraffin processing and the other imersed in Clark's fixative for microdissection. Intestinal architecture was evaluated by measuring villi height and widht and crypt depth in both methods. The results show that measurements of villi and crypts in histological sections were consistently shorter than those obtained in microdissection technique. Microdissection method give accurate information and insight into the relative sizes and shapes of villi and crypts, does not causes retraction artefacts related with dehydration, but as a limited time of execution and observation, while HE preparations are almost eternals. Besides that, HE preparations are also suitable for pathologic diagnosis. In conclusion we think that the microdissection method complement the conventionally haematoxilin and eosin method.

Key words: small intestine, rabbit, histomorphometry, ileal mucosa.

INTRODUCTION

In rabbit, the incidence of digestive troubles is higher after weaning. The mucosal epithelium of small intestine serves as a delicate interface between external and internal environments, and its primary function is to digest and absorb dietary nutrients. To examine fully intestinal architecture information is needed on shapes and sizes of villi and crypts and also their heterogenecity. Individual villi and crypts from intestinal tissue have been dissected and their shape, sizes, and properties examined in many investigations since the first quantitative study of morphology in small intestine in 1948 (LEBLOND and STEVENS, 1948). We have adapted the microdissection technique, described by CLARKE (1977), to samples from rabbit small intestine. This work describes this technique and its application to the measurement of villi and crypts in 24 specimens of morphologically normal ileum tissue from 24 rabbits at three different ages (21 days, 32 and 42) and the results are compared with those obtained by the measuring sections of the same specimen stained with haematoxylin and eosin.

MATERIAL AND METHODS

Twenty-four rabbits, previously brooded under identical conditions with the diet described in table 1 and 2, were used. The animals were slaughtered at 24 (n=8), 32 (n=8) and 42 (n=8) days of age to collect samples for laboratory analysis.

Table 1. Composition of basal diet (g/kg of diet).

Dehydrated alfafa 14/15	233.3	L-lysine	1.6
Sunflower meal	176.0	Methionine hidroxy analogue (MHA)	0.5
Wheat	106.0	Salt	3
Beet pulp	98	Monocalcicum phosphate 22,6%	10.6
Molasse	60.0	Exal- H	20.0
Citrine pulp	60.0	PX9520 5REEAP (rabbit)	5
Corn distiller's grain dehydrated	30.0	Nutacid B2	5
Wheat bran	49.0	Racikern B2/ Sant BZ	2
Grape-seed meal	37.0		
Sunflower hulls	20.0		
Animal fat	8.0		
Sunflower oil	5		

Table 2. Estimated nutritive value of basal diet.

Dry matter ($g.kg^{-1}$ feed)	898.9	Crude fibre ($g.kg^{-1}$ feed)	167
Protein ($g.kg^{-1}$ feed)	142.5	ADF($g.kg^{-1}$ feed)	217.0
Fat ($g.kg^{-1}$ feed)	35	NDF($g.kg^{-1}$ feed)	343.3
Sugar ($g.kg^{-1}$ feed)	66.9	Lenhin ($g.kg^{-1}$ feed)	60.0
Starch ($g.kg^{-1}$ feed)	120.0	Digestible energy ($kcal.kg^{-1}$ feed)	2356
Ash ($g.kg^{-1}$ feed)	95.4		

The small intestine was dissected free of its mesentery immediately after slaughter. Two segments from ileum, with 2 cm each, were cut longitudinally at the mesenteric attachment. Samples for light microscopy were immediately fixed in 10% neutral formalin. Tissues were processed in an automatic tissue processor (Shandon – Hipercenter XP) and embedded in paraffin wax (Histoplast – Shandon). Three-micrometer paraffin sections were cut on a Leica Jung Biocut 2035 microtome and routinely stained with haematoxylin and eosin (HE) for histological examination.

Samples for microdissection were attached to a piece x-ray film, villi upwards, left for 60-90 seconds to adhere, and then immersed in Clark's fixative (75% ethyl alcohol, 25% glacial acetic acid). After 3-24 hours at room temperature the samples were transferred to 75% ethyl alcohol in water and stored, not longer than 8 weeks. The samples were stained in bulk by Feulgen reaction and, in tap water, were examined with a dissecting microscope, using fine forceps and a cataract knife. A number of strips1.5-3mm long and ±1 villus thick was cut free. These strips were placed on a slide in a drop of 45% acetic acid and a coverslip was applied.

Slides from both methods were evaluated on a Nikon Eclipse E600 microscope and digitised using video image software (ACT-1 for the Nikon DXM1200 digital still camera). Images were analysed (optical lens No 4) to measure the crypt depth, villi height and width of the villi at the crypt/villus junction as well as the tip. Fifteen villi were assessed per sample and the reported mean value was based in these measurements. The measurements were determined according to a modification of the method by JAEGER *et al.* (1990) and IJI *et al.* (2001).

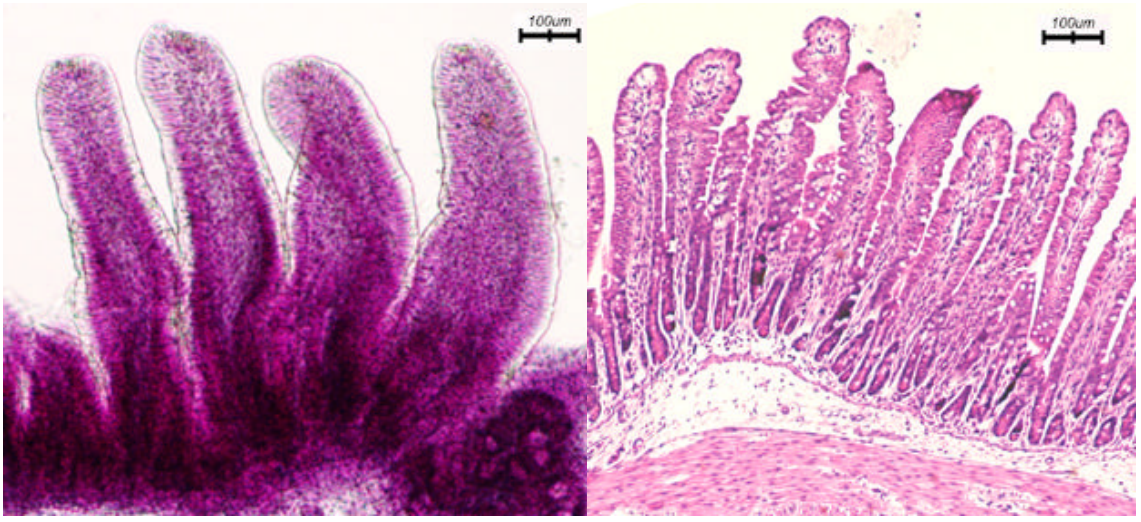

Figure 1. Rabbit ileal mucosa: microdissected, Feulgen-reaction stained, (left); HE (right).

RESULTS AND DISCUSSION

The results show that measurements of villi and crypts in histological sections were consistently shorter than those obtained in microdissection technique (table 3, figure 2 and 3).

Table 3. Results of measurements (?m) at different ages and coefficient of variation (CV %).

	Microdissection						Haematoxylin and eosin					
	24 days		32 days		46 days		24 days		32 days		46 days	
	µm	CV	µm	CV	µm	CV	µm	CV	µm	CV	µm	CV
Villus height	610	15	564	18	608	15	436	18	392	18	403	9
Villus width at the tip	136	12	134	10	185	17	72	17	75	11	93	15
Villus width at the crypt/villus junction	135	26	213	15	267	15	73	8	92	19	123	11
Crypt depth	212	20	205	26	181	17	85	14	123	23	140	7
Villus height/ Crypt depth	2.95	20	2.95	35	3.38	10	5.22	24	3.17	26	2.89	15

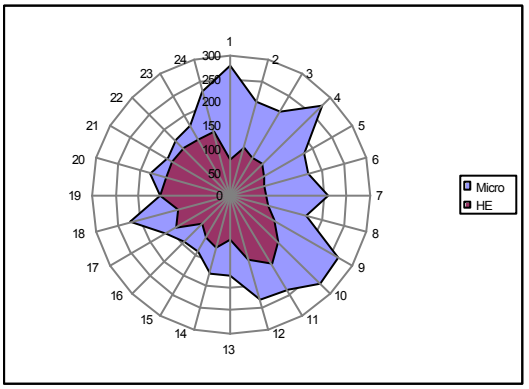

Figure 2. Measurements of crypts examine by conventional histology (HE) and microdissection (Micro)

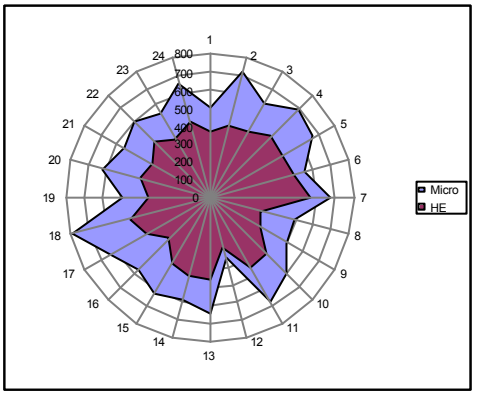

Figure 3. Measurements of Villi height examine by conventional histology (HE) and microdissection (Micro).

The microdissection technique is rapid, sensitive and inexpensive. It gives accurate information and insight into the relative sizes and shapes of villi and crypts (BODE *et al.,* 1982, GOODLAD *et al*., 1991). The results show that measurements of villi and crypts in histological sections were consistently shorter than those obtained in microdissection technique which is in according with other works (FERGUSON *et al.,* 1977). The variations observed regarding to crypts depth, that in the microdissection technique diminish with age and in HE method increase, is probably due to tissue characteristics (in young animals, they are more loose than in the older which leads to a bigger retraction during processing) and also because of the tissue orientation in paraffin inclusion (it is not always totally perpendicular). Microdissection is quicker and less expensive, does not

causes retraction artefacts related with dehydration, but as a limited time of execution and observation, while HE preparations are almost eternal. Besides that, HE preparations are also suitable for pathologic diagnosis. The population of enterocytes is in a dynamic state, constantly being replaced by regeneration of epithelial cells from crypts and the rate of regeneration matches the normal loss of villus epithelium (TANG *et al*., 1999). The epithelial cells near the villus tip are the matures and have the greatest digestive and absorptive capacity. In general, measurements of villus height and crypt depth give an indication of the likely maturity and functional capacity of enterocytes.

CONCLUSIONS

In conclusion we think that the microdissection method complements the conventionally haematoxylin and eosin method. It is a simple, rapid and inexpensive method, which gives accurate information on villi morphometry, but does not allow histopathological diagnosis and has a shorter time of observation. Further studies will be needed to clarify and correlate the differences between measurements by both methods.

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